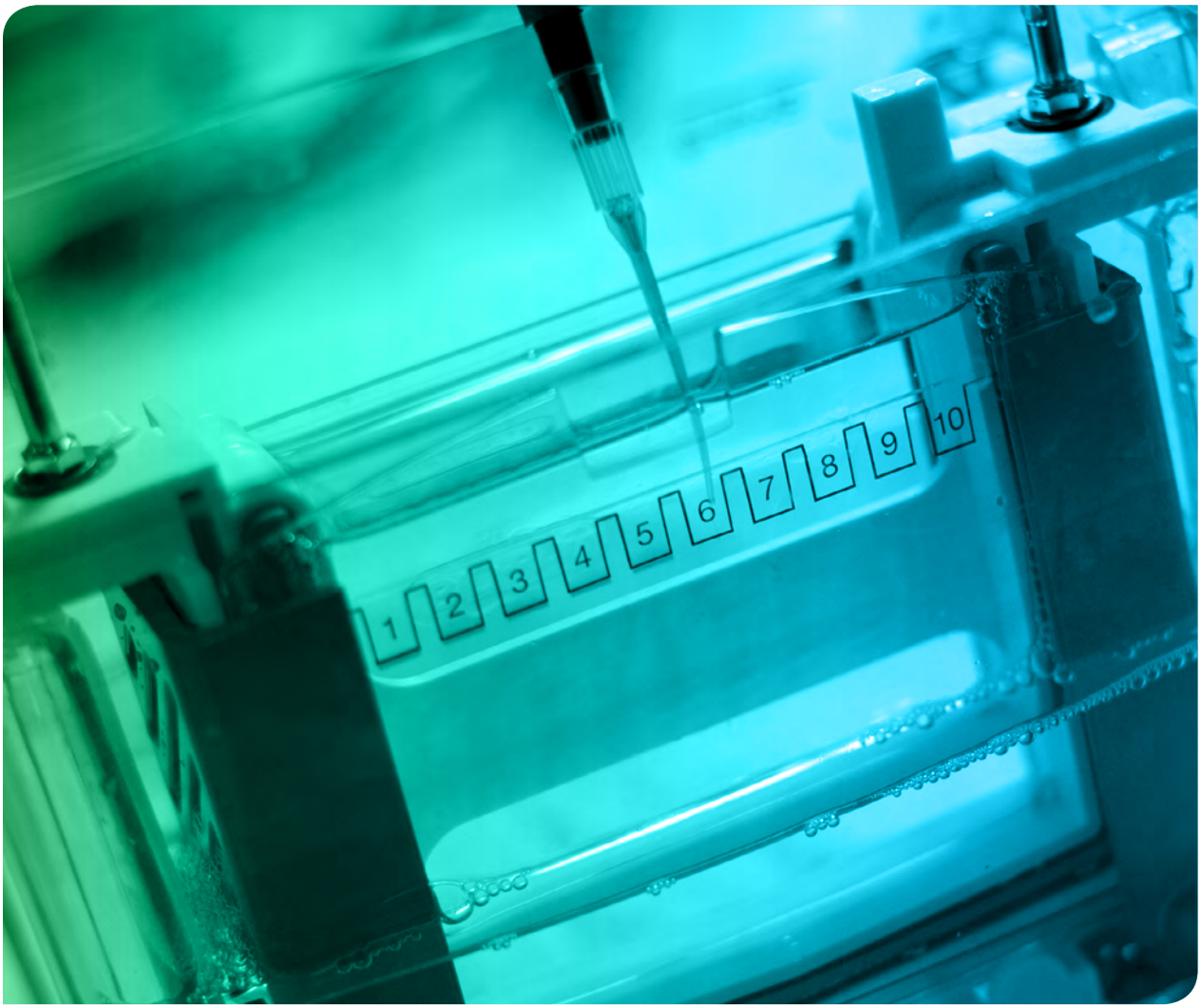

A Guide to Polyacrylamide Gel Electrophoresis and Detection

BIO-RAD

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PART I
Theory and Product Selection

CHAPTER 1
Overview

Protein electrophoresis is the movement of proteins within an electric field. Popular and widely used in research, it is most commonly used to separate proteins for the purposes of analysis and purification. This chapter provides a brief overview of the theory and workflow behind protein electrophoresis.

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How Protein Electrophoresis Works

The term electrophoresis refers to the movement of charged molecules in response to an electric field, resulting in their separation.

In an electric field, proteins move toward the electrode of opposite charge. The rate at which they move (migration rate, in units of cm^2/Vsec) is governed by a complex relationship between the physical characteristics of both the electrophoresis system and the proteins. Factors affecting protein electrophoresis include the strength of the electric field, the temperature of the system, the pH, ion type, and concentration of the buffer as well as the size, shape, and charge of the proteins (Garfin 1990) (Figure 1.1). Proteins come in a wide range of sizes and shapes and have charges imparted to them by the dissociation constants of their constituent amino acids. As a result, proteins have characteristic migration rates that can be exploited for the purpose of separation. Protein electrophoresis can be performed in either liquid or gel-based media and can also be used to move proteins from one medium to another (for example, in blotting applications).

Over the last 50 years, electrophoresis techniques have evolved as refinements have been made to the buffer systems, instrumentation, and visualization techniques used. Protein electrophoresis can be used for a variety of applications such as purifying proteins, assessing protein purity (for example, at various stages during a chromatographic separation), gathering data on the regulation of protein expression, or determining protein size, isoelectric point (pI), and enzymatic activity. In fact, a significant number of techniques including gel electrophoresis, isoelectric focusing (IEF), electrophoretic transfer (blotting), and two-dimensional (2-D) electrophoresis can be grouped under the term “protein electrophoresis” (Rabilloud 2010). Though some information is provided about these methods in the following chapters, this guide focuses on the one-dimensional separation of proteins in polyacrylamide gels, or polyacrylamide gel electrophoresis (PAGE).

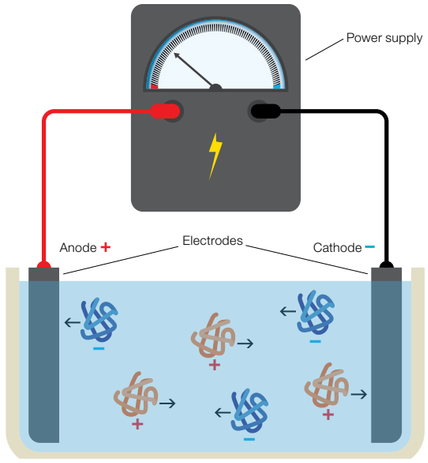

Fig. 1.1. Movement of proteins during electrophoresis.

General Considerations and Workflow

The electrophoresis workflow (Figure 1.2) involves the selection of the appropriate method, instrumentation, and reagents for the intended experimental goal. Once proteins are separated, they are available for a number of downstream applications, including enzymatic assays, further purification, transfer to a membrane for immunological detection (immunoblotting or western blotting), and elution and digestion for mass spectrometric analysis.

Related Literature

Protein Blotting Guide, A Guide to Transfer and Detection, bulletin 2895

2-D Electrophoresis for Proteomics: A Methods and Product Manual, bulletin 2651

Protein Electrophoresis Workflow

Method Selection

Consider the experimental goals in selecting the appropriate electrophoresis method. Instrumentation selection depends on the desired resolution and throughput.

Sample Preparation

The protein sample may be prepared from a biological sample, or it may come from a step in a purification workflow. In either case, prepare the protein at a concentration and in a buffer suitable for electrophoresis.

Gel and Buffer Preparation

Whether handcast or precast, the gel type used should suit the properties of the protein under investigation, the desired analysis technique, and overall goals of the experiment. Buffer selection depends on the gel type and type of electrophoresis performed.

Performing Electrophoresis

Gels are placed in the electrophoresis cell, buffer is added, and samples are loaded. Select running conditions that provide optimum resolution while maintaining the temperature of the system during separation.

Protein Detection and Analysis

Select a visualization technique that matches sensitivity requirements and available imaging equipment.

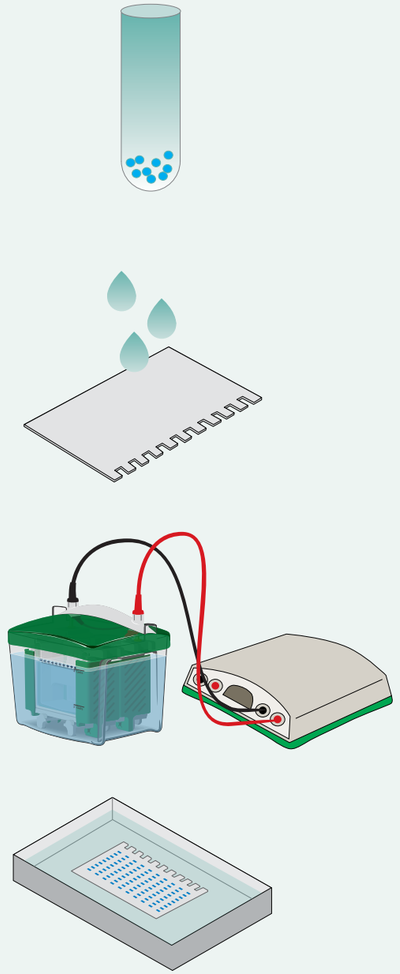

Fig. 1.2. Protein electrophoresis workflow.

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CHAPTER 2

Protein Electrophoresis Methods and Instrumentation

Consider the experimental goals in selecting the appropriate electrophoresis method; selection of instrumentation depends on the number and volume of samples, desired resolution, and throughput. This chapter describes the most common techniques and systems in use today.

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Protein Electrophoresis Methods

By choosing suitable separation matrices and corresponding buffer systems, a range of experimental objectives can be met using protein electrophoresis (Zewart and Harrington 1993).

Polyacrylamide Gel Electrophoresis (PAGE)

When electrophoresis is performed in acrylamide or agarose gels, the gel serves as a size-selective sieve during separation. As proteins move through a gel in response to an electric field, the gel's pore structure allows smaller proteins to travel more rapidly than larger proteins (Figure 2.1). For protein separation, virtually all methods use polyacrylamide as an anticonvective, sieving matrix covering a protein size range of 5–250 kD. Some less common applications such as immunoelectrophoresis and the separation of large proteins or protein complexes >300 kD rely on the larger pore sizes of agarose gels.

In most PAGE applications, the gel is mounted between two buffer chambers, and the only electrical path between the two buffers is through the gel. Usually, the gel has a vertical orientation, and the gel is cast with a comb that generates wells in which the samples are applied (Figure 2.1). Applying an electrical field across the buffer chambers forces the migration of protein into and through the gel (Hames 1998).

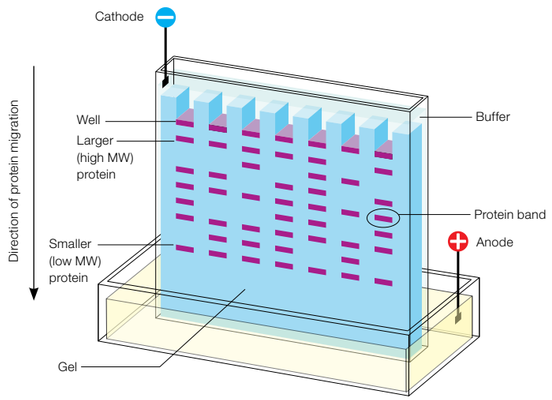

Fig. 2.1. Schematic of electrophoretic protein separation in a polyacrylamide gel. MW, molecular weight.

Two types of buffer systems can be used:

- Continuous buffer systems use the same buffer (at constant pH) in the gel, sample, and electrode reservoirs (McLellan 1982). Continuous systems are not common in protein separations; they are used mostly for nucleic acid analysis
- Discontinuous buffer systems use a gel separated into two sections (a large-pore stacking gel on top of a small-pore resolving gel, Figure 2.2) and different buffers in the gels and electrode solutions (Wheeler et al. 2004)

In gel electrophoresis, proteins do not all enter the gel matrix at the same time. Samples are loaded into wells, and the proteins that are closer to the gel enter the gel first. In continuous systems, the uniform separation matrix yields protein bands that are diffuse and poorly resolved. In discontinuous systems, on the other hand, proteins first migrate quickly through the large-pore stacking gel and then are slowed as they enter the small-pore resolving gel. As they slow down, they stack on top of one another to form a tight band, which improves resolution. Discontinuous systems also use ions in the electrophoresis buffer that sandwich the proteins as they migrate through the gel, and this tightens the protein bands even more (Figure 2.2). Discontinuous buffer systems provide higher resolution than continuous systems, and varying the buffers used in the sample, gel, and electrode chambers creates a variety of discontinuous buffer systems that can be used for a variety of applications.

Discontinuous Native PAGE

The original discontinuous gel system was developed by Ornstein and Davis (Ornstein 1964, Davis 1964) for the separation of serum proteins in a manner that preserved native protein conformation, subunit interactions, and biological activity (Vavricka 2009). In such systems, proteins are prepared in nonreducing, nondenaturing sample buffer, and electrophoresis is also performed in the absence of denaturing and reducing agents.

Data from native PAGE are difficult to interpret. Since the native charge-to-mass ratio of proteins is preserved, protein mobility is determined by a complex combination of factors. Since protein-protein interactions are retained during separation, some proteins may also separate as multisubunit complexes and move in unpredictable ways. Moreover, because native charge is preserved, proteins can migrate towards either electrode, depending on their charge. The result is that native PAGE yields unpredictable separation patterns that are not suitable for molecular weight determination.

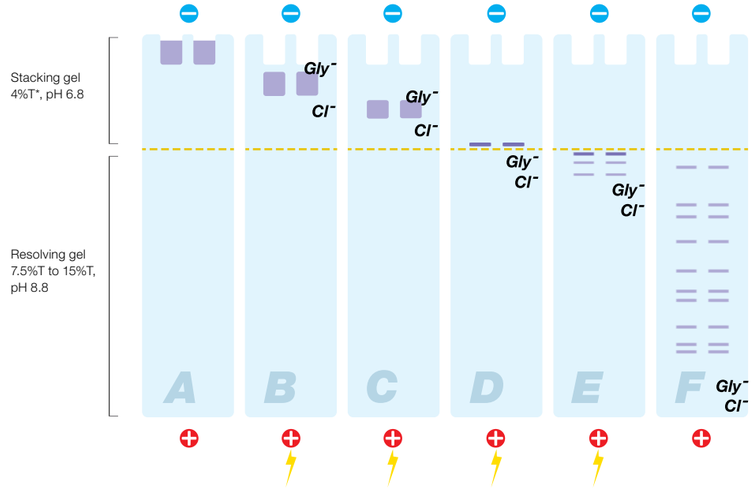

Fig. 2.2. Migration of proteins and buffer ions in a denaturing discontinuous PAGE system. A, Denatured sample proteins are loaded into the wells; **B**, Voltage is applied and the samples move into the gel. The chloride ions already present in the gel (leading ions) run faster than the SDS-bound proteins and form an ion front. The glycinate ions (trailing ions) flow in from the running buffer and form a front behind the proteins; **C**, A voltage gradient is created between the chloride and glycinate ions, which sandwich the proteins in between them; **D**, The proteins are stacked between the chloride and glycinate ion fronts. At the interface between the stacking and resolving gels, the percentage of acrylamide increases and the pore size decreases. Movement of the proteins into the resolving gel is met with increased resistance; **E**, The smaller pore size resolving gel begins to separate the proteins based on molecular weight only, since the charge-to-mass ratio is equal in all the proteins of the sample; **F**, The individual proteins are separated into band patterns ordered according to their molecular weights.

* %T refers to the total monomer concentration of the gel (see Chapter 4 for more information).

Nevertheless, native PAGE does allow separation of proteins in their active state and can resolve proteins of the same molecular weight.

SDS-PAGE

To overcome the limitations of native PAGE systems, Laemmli (1970) incorporated the detergent sodium dodecyl sulfate (SDS) into a discontinuous denaturing buffer system, creating what has become the most popular form of protein electrophoresis, SDS-PAGE.

When proteins are separated in the presence of SDS and denaturing agents, they become fully denatured and dissociate from each other. In addition, SDS binds noncovalently to proteins in a manner that imparts:

- An overall negative charge on the proteins. Since SDS is negatively charged, it masks the intrinsic charge of the protein it binds
- A similar charge-to-mass ratio for all proteins in a mixture, since SDS binds at a consistent rate of 1.4 g of SDS per 1 g protein (a stoichiometry of about one SDS molecule per two amino acids)
- A long, rod-like shape on the proteins instead of a complex tertiary conformation (Figure 2.3)

As a result, the rate at which SDS-bound protein migrates in a gel depends primarily on its size, enabling molecular weight estimation.

The original Laemmli system incorporated SDS in the gels and buffers, but SDS is not required in the gel. SDS in the sample buffer is sufficient to saturate proteins, and the SDS in the cathode buffer maintains the SDS saturation during electrophoresis. Precast gels (manufactured gels such as Bio-Rad's Mini-PROTEAN® and Criterion™ Gels) do not include SDS and so can be used for either native or SDS-PAGE applications. A range of gel and buffer combinations can be used for native and SDS-PAGE, each with its own advantages (see Chapter 4 for more details).

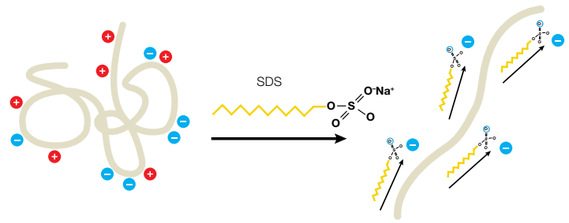

Fig. 2.3. Effect of SDS on the conformation and charge of a protein.

Related Literature

Gel Electrophoresis: Separation of Native Basic Proteins by Cathodic, Discontinuous Polyacrylamide Gel Electrophoresis, bulletin 2376

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Other Types of PAGE

Blue Native PAGE (BN-PAGE)

BN-PAGE is used to separate and characterize large protein complexes in their native and active forms. Originally described by Schägger and von Jagow (1987), this technique relies on the solubilization of protein complexes with mild, neutral detergents and the binding of negatively charged Coomassie (Brilliant) Blue G-250 Stain to their surfaces. This imparts a high charge-to-mass ratio that allows the protein complexes to migrate to the anode as they do in SDS-PAGE. Coomassie Blue does not, however, denature and dissociate protein complexes the way SDS does. High-resolution separation is achieved by electrophoresis into an acrylamide gradient with decreasing pore sizes; the protein complexes become focused at the corresponding pore size limit (Nijtmans et al. 2002, Reisinger and Eichacker 2008).

Zymogram PAGE

Zymogram PAGE is used to detect and characterize collagenases and other proteases within the gel. Gels are cast with gelatin or casein, which acts as a substrate for the enzymes that are separated in the gel under nonreducing conditions. The proteins are run with denaturing SDS in order to separate them by molecular weight. After renaturing the enzymes and then allowing them to break down the substrate, zymogram gels are stained with Coomassie (Brilliant) Blue R-250 Stain, which stains the substrate while leaving clear areas around active proteases.

Isoelectric Focusing (IEF)

IEF combines the use of an electric field with a pH gradient to separate proteins according to their pI. It offers the highest resolution of all electrophoresis techniques (Westermeier 2004).

When a protein moves through a pH gradient, its net charge changes in response to the pH it encounters. Under the influence of an electric field, a protein in a pH gradient migrates to a pH where its net charge is zero (the protein's pI). If the protein moves out of that position, it acquires a charge and is forced back to the zero-charge position (Figure 2.4). This focusing is responsible for the high resolution of IEF. pI values of proteins usually fall in the range of pH 3–11.

Related Literature

2-D Electrophoresis for Proteomics: A Methods and Product Manual, bulletin 2651

Links

Coomassie Stains

Coomassie Brilliant Blue G-250 Stain

Coomassie Brilliant Blue R-250 Stain

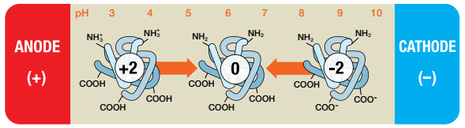

Fig. 2.4. Isoelectric focusing. A protein is depicted in a pH gradient in an electric field. A pH gradient formed by ampholyte molecules under the influence of an electric field is indicated. The gradient increases from acidic (pH 3) at the anode to basic (pH 10) at the cathode. The hypothetical protein in the drawing bears a net charge of +2, 0, or –2, at the three positions in the pH gradient shown. The electric field drives the protein toward the cathode when it is positively charged and toward the anode when it is negatively charged, as shown by the arrows. At the pI, the net charge on the protein is zero, so it does not move in the field. The protein loses protons as it moves toward the cathode and becomes progressively less positively charged. Conversely, the protein gains protons as it moves toward the anode and becomes less negatively charged. When the protein becomes uncharged (pI), it ceases to move in the field and becomes focused.

Two methods are used to generate a stable, continuous pH gradient between the anode and cathode:

- Carrier ampholytes — heterogeneous mixtures of small (300–1,000 Da) conductive polyamino-polycarboxylate compounds that carry multiple charges with closely spaced pI values. When voltage is applied across an ampholyte-containing solution or gel, the ampholytes align themselves according to their pIs and buffer the pH in their proximity, establishing a pH gradient. Ampholytes can be used in gels (for example, tube gels or vertical gels) or in solution (for example, liquid-phase IEF)
- Immobilized pH gradients (IPG) strips — formed by covalently grafting buffering groups to a polyacrylamide gel backbone. A gradient of different buffering groups generates a stable pH gradient that can be tailored for different pH ranges and gradients (Bjellquist et al. 1982)

Bio-Rad's PROTEAN® i12™ IEF System provides individual lane control for up to 12 IPG strips, making it possible to run different sample types, different pH gradients, and multiple protocols at the same time. IEF can be run under either native or denaturing conditions. Native IEF retains protein structure and enzymatic activity. However, denaturing IEF is performed in the presence of high concentrations of urea, which dissociates proteins into individual subunits and abolishes secondary and tertiary structures. Whereas native IEF may be a more convenient option because it can be performed with a variety of precast gels, denaturing IEF often offers higher resolution and is more suitable for the analysis of complex protein mixtures.

2-D Electrophoresis

The sequential application of different electrophoresis techniques produces a multi-dimensional separation. The most common 2-D technique (O'Farrell 1975) subjects protein samples first to denaturing IEF on a tube gel or IPG gel strip (for separation by pI), then to SDS-PAGE for further separation by molecular weight. High-resolution 2-D methods enable separation of thousands of polypeptides in a single slab gel. The resulting spots can be visualized by gel staining, or they can be transferred to a membrane support for total protein staining or analysis with specific antibody detection. For more details, refer to 2-D Electrophoresis for Proteomics (bulletin 2651).

Electrophoresis Cells and Power Supplies

Electrophoresis Cells

Vertical electrophoresis cells are plastic boxes with anode and cathode buffer compartments that contain electrodes (Figure 2.5). The electrodes (typically platinum wire) connect to a jack attached to a power supply. The gels are held vertically between the electrode chambers during electrophoresis (Andrews 1986).

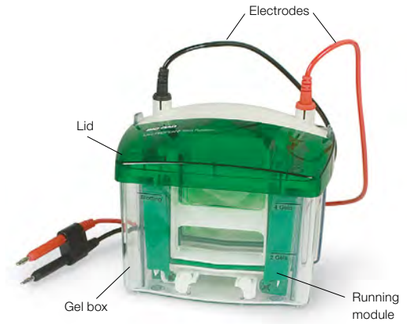

Fig. 2.5. Components of a vertical electrophoresis cell.

Vertical electrophoresis cells are made in different size formats to accommodate different gels sizes. Deciding which cell to use depends on the requirements for speed, resolution, and throughput (both the number of samples and gels) as well as the volume of sample available (Table 2.1).

- Mini-format systems — accommodate small gels (up to 8.6 x 6.7 cm). The short separation distance maximizes the electrical field strength (V/cm) to yield rapid separations with moderate resolution. Use these systems for rapid analysis, method development, or when sample volumes are limited. The Mini-PROTEAN® System includes the Mini-PROTEAN Tetra Cell (with a capacity of up to four gels) and the high-throughput Mini-PROTEAN® 3 Dodeca™ Cell (for running up to 12 gels); both cells are compatible with Mini-PROTEAN Precast Gels
- Midi-format systems — accommodate 13.3 x 8.7 cm gels and offer rapid runs with more samples per gel and enhanced separation over mini-format gels. The Criterion™ System includes the Criterion Cell (for 1–2 gels) and the high-throughput Criterion™ Dodeca™ Cell (for 1–12 gels); both cells are compatible with Criterion Precast Gels
- Large-format systems — accommodate large gels (up to 20 x 18.3 cm for the PROTEAN® II System and 20 x 20.5 cm for the PROTEAN Plus System) and offer maximum resolution. The PROTEAN II System provides a choice of glass plates, spacer, and sandwich clamps to cast two gel lengths: 16 or 20 cm. The PROTEAN® Plus Dodeca™ Cell allows maximum throughput with the capability to run up to 12 gels at a time

Related Literature

Mini-PROTEAN Tetra Cell Brochure, bulletin 5535

Criterion Precast Gel System Brochure, bulletin 2710

PROTEAN II xi/XL Cells Product Information Sheet, bulletin 1760

Links

Mini Format 1-D Electrophoresis Systems

Mini-PROTEAN Precast Gels

Mini-PROTEAN Tetra Cell

Mini-PROTEAN 3 Dodeca Cell

Midi Format 1-D Electrophoresis Systems

Criterion Precast Gels

Criterion Cell

Criterion Dodeca Cell

Large-Format 1-D Electrophoresis Systems

PROTEAN II xi Cell

PROTEAN II XL Cell

PROTEAN II xi and XL Multi-Cells

PROTEAN Plus Dodeca Cell

PROTEAN i12 IEF System

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Table 2.1. Vertical electrophoresis system selection guide.

	Mini-PROTEAN System	Criterion System	PROTEAN II System	PROTEAN Plus System
				
Advantages	Run 1–4 precast or handcast gels in the Mini-PROTEAN Tetra Cell and up to 12 gels in the Mini-PROTEAN Dodeca Cell in mini format Wing clamp assembly allows faster setup and leak-free operation	Fast setup with drop-in gel and cell design (precast or handcast) Run 1–2 precast Criterion or handcast gels in the Criterion Cell and up to 12 gels in the Criterion Dodeca Cell Integrated upper buffer chamber allows leak-free operation	Large-format gel system offers greater resolution over smaller formats Can accommodate up to 4 gels and is available in xi or XL formats for running a variety of gel sizes Multi-cell is available for running up to 6 gels	Offers maximum resolution in a single gel and the longest range of separation (with the ability to run up to 12 gels) Specifically for the second dimension of 2-D electrophoresis
Compatible Gel Formats				
Precast	Mini-PROTEAN Precast Gels Ready Gel® Precast Gels	Criterion Precast Gels		
Handcast	Ready Gel Empty Cassettes Mini-PROTEAN Casting Plates	Criterion Empty Cassettes	PROTEAN II Casting Plates	PROTEAN Plus Casting Equipment
Electrophoresis Cells				
	Mini-PROTEAN Tetra Mini-PROTEAN 3 Dodeca	Criterion Criterion Dodeca	PROTEAN II xi/XL PROTEAN II xi/XL Multi-Cells	PROTEAN Plus Dodeca
Precast Gel Dimensions				
W x L x thickness	Mini-PROTEAN Precast Gels: 8.6 x 6.7 x 0.1 cm Ready Gel Precast Gels: 8.3 x 6.4 x 0.1 cm	Criterion Precast Gels: 13.3 x 8.7 x 0.1 cm		
Cassette Dimensions (for Handcasting Gels)				
	10.0 x 8.0 cm	15.0 x 10.6 cm	20.0 x 18.3 cm	18.5 x 20.5 cm 20.0 x 20.5 cm 20.0 x 20.5 cm
Compatible Transfer Systems				
Wet/tank transfer	Mini Trans-Blot® Cell Criterion Blotter Trans-Blot® Cell	Criterion Wire Blotter Criterion Plate Blotter Trans-Blot Cell Trans-Blot Plus Cell	Trans-Blot Cell Trans-Blot Plus Cell	Trans-Blot Plus Cell
Semi-dry transfer	Trans-Blot® Turbo™ System Trans-Blot SD Cell	Trans-Blot Turbo System Trans-Blot SD Cell	Trans-Blot SD Cell	

Power Supplies for PAGE Applications

Power supplies are available to meet the power requirements of numerous applications. The choice of power supply for PAGE applications usually depends on the size and number of gels being run. Table 2.2 compares the Bio-Rad PowerPac Power Supplies recommended for vertical electrophoresis applications.

Table 2.2. PowerPac™ Power Supplies selection guide.

Technique and Recommended Apparatus	PowerPac Power Supply
Laemmli (SDS), O'Farrell Second Dimension (SDS)	
Mini-PROTEAN Tetra Cell	Basic or HC
Criterion Cell	Basic or HC
PROTEAN II xi Cell	HV or Universal
PROTEAN II XL Cell	HV or Universal
High-Throughput Electrophoresis	
Mini-PROTEAN 3 Dodeca Cell	HC or Universal
Criterion Dodeca Cell	HC or Universal
PROTEAN II xi/XL Multi-Cell	Universal
PROTEAN Plus Dodeca Cell	HC or Universal
Western Blotting	
Mini Trans-Blot Cell	HC
Criterion Blotter	
Wire electrodes	HC
Plate electrodes	HC
Trans-Blot Cell	
Wire electrodes	HC
Plate electrodes	HC
High-intensity transfer	HC
Trans-Blot Plus Cell	HC
Trans-Blot SD Cell	
Protein	HC
DNA/RNA	HC

- Use the PowerPac Basic or PowerPac HC High-Current Power Supply for mini-format vertical PAGE applications
- Use the PowerPac HV High-Voltage or PowerPac Universal Power Supply for large-format vertical PAGE applications
- Use the PowerPac HC Power Supply for applications that require high currents, such as PAGE with the high-throughput Dodeca Cells


PowerPac HC High-Current Power Supply


PowerPac Basic Power Supply


PowerPac Universal Power Supply


PowerPac HV High-Voltage Power Supply

Fig. 2.6. PowerPac Power Supplies.

Preparative Electrophoresis

Preparative electrophoresis techniques separate large amounts of protein (nanogram to gram quantities) for the purposes of purification or fractionation (to reduce sample complexity). The same principles that are applied for analytical work can be applied for preparative work.

PAGE

Preparative PAGE can be accomplished using a standard slab gel or special instrumentation. With the slab gel, a single preparative or "prep" well is cast, which allows a large volume of a single sample to be applied within one well. With this approach, the separated protein is retained within the gel for further analysis or purification (for example, by electroelution).

Alternatively, continuous-elution gel electrophoresis using the Model 491 Prep Cell or Mini Prep Cell yields high-resolution separations and proteins in liquid fractions, ready for downstream use.

Combination Approaches (2-D Separations)

Preparative IEF and PAGE can be combined (for separation on multiple dimensions) for even greater separation.

Related Literature

PowerPac Basic 300 V Power Supply Flier, bulletin 2881

PowerPac HC High-Current Power Supply Flier, bulletin 2882

PowerPac Universal Power Supply Brochure, bulletin 2885

PowerPac HV Power Supply Brochure, bulletin 3189

Links

Preparative Electrophoresis
Power Supplies
PowerPac Universal Power Supply
PowerPac HC High-Current Power Supply
PowerPac HV High-Voltage Power Supply
PowerPac Basic Power Supply
Model 491 Prep Cell and Mini Prep Cell

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CHAPTER 3

Sample Preparation for Electrophoresis

Sample preparation involves the extraction and solubilization of a protein sample that is free of contaminants and that has a total protein concentration suitable for electrophoresis. The quality of sample preparation can greatly affect the quality of the data that are generated. General guidelines and some of the most common methods for protein sample preparation are provided in this chapter.

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Sample Preparation Workflow

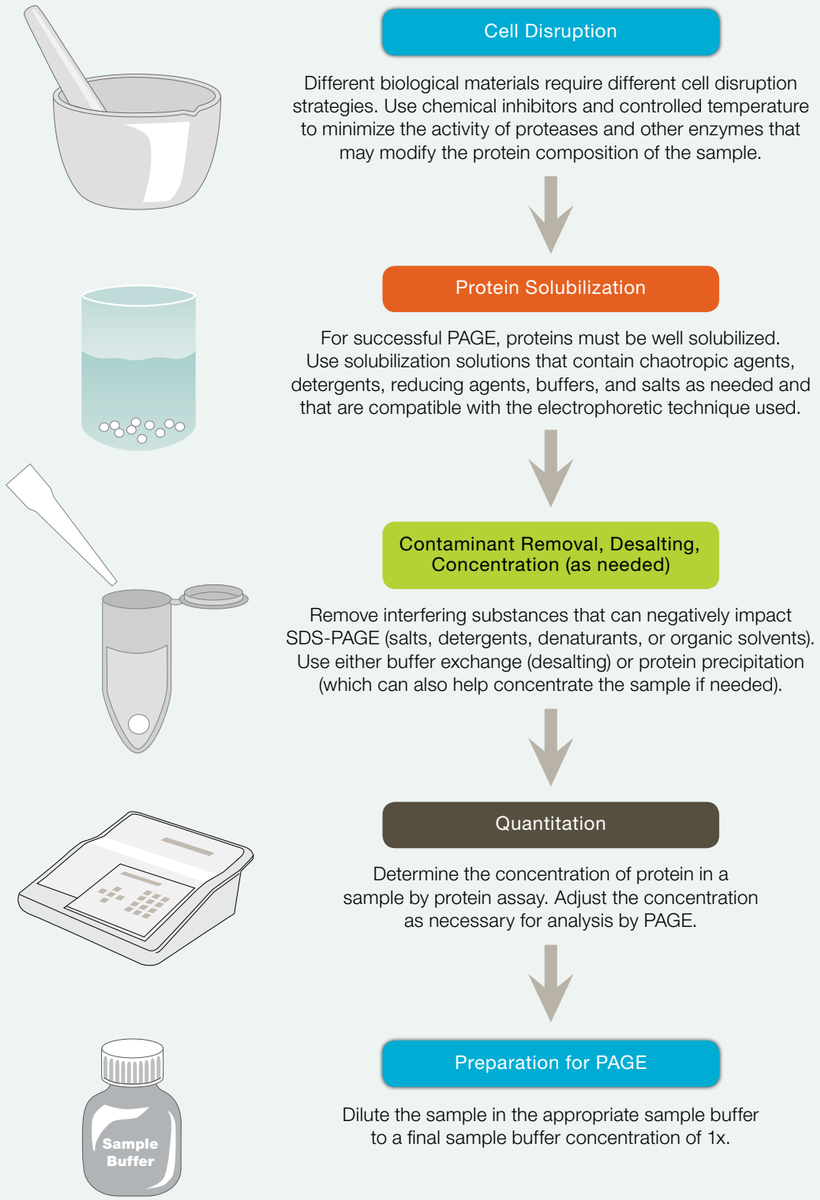

Fig. 3.1. Protein sample preparation workflow.

General Considerations

Due to the great diversity of protein sample types and sources, no single sample preparation method works with all proteins; for any sample, the optimum procedure must be determined empirically. However, the following general sample preparation guidelines should be kept in mind to avoid a number of common pitfalls during sample preparation for protein electrophoresis (Posch et al. 2006):

- Keep the sample preparation workflow as simple as possible (increasing the number of sample handling steps may increase variability)
- With cell or tissue lysates, include protease inhibitors to minimize artifacts generated by proteolysis; protease inhibitors are generally not required for samples like serum or plasma
- Determine the amount of total protein in each sample using a protein assay that is compatible with chemicals in your samples
- Solubilize proteins in a buffer that is compatible with the corresponding electrophoresis technique
- Use protein extracts immediately or aliquot them into appropriately sized batches and store them at –80°C to avoid freeze-thaw cycles

Cell Disruption

The effectiveness of a cell disruption method determines the accessibility of intracellular proteins for extraction and solubilization (Huber et al. 2003). Different biological materials require different cell disruption strategies, which can be divided into two main categories: gentle and harsher methods (Table 3.1).

- Use gentle cell disruption protocols when the sample consists of cells that lyse easily, such as red blood cells or tissue culture cells
- Use harsher methods, which are based mainly on mechanical rupture (Goldberg 2008), with biological materials that have tough cell walls (for example, plant cells, tissues, and some microbes)
- When working with a new sample, use at least two different cell disruption protocols and compare their efficiency in terms of yield (by protein assay) and qualitative protein content (by SDS-PAGE)
- Optimize the power settings of mechanical rupture systems and incubation times for all lysis approaches
- Mechanical cell lysis usually generates heat; use cooling where required to avoid overheating the sample

Table 3.1. Suitability of cell disruption methods to various sample types.

Technique	Description	Bacteria	Yeast, Algae, Fungi	Seeds	Green Plant Material	Soft Tissues	Mammalian Cell Culture
Gentle Methods							
Osmotic lysis	Suspension of cells in hypotonic solution; cells swell and burst, releasing cellular contents	–	–	–	–	–	•
Freeze-thaw lysis	Freezing in liquid nitrogen and subsequent thawing of cells	–	–	–	–	–	•
Detergent lysis	Suspension of cells in detergent-containing solution to solubilize the cell membrane; this method is usually followed by another disruption method, such as sonication	–	–	–	–	–	•
Enzymatic lysis	Suspension of cells in iso-osmotic solutions containing enzymes that digest the cell wall (for example, cellulase and pectinase for plant cells, lyticase for yeast cells, and lysozyme for bacterial cells); this method is usually followed by another disruption method, such as sonication	•	•	–	•	–	–
Harsher Methods							
Sonication	Disruption of a cell suspension, cooled on ice to avoid heating and subjected to short bursts of ultrasonic waves	•	•	–	–	–	•
French press	Application of shear forces by forcing a cell suspension through a small orifice at high pressure	•	•	–	–	–	•
Grinding	Breaking cells of solid tissues and microorganisms with a mortar and pestle; usually, the mortar is filled with liquid nitrogen and the tissue or cells are ground to a fine powder	•	•	•	•	•	–
Mechanical homogenization	Homogenization with either a handheld device (for example, Dounce and Potter-Elvehjem homogenizers), blenders, or other motorized devices; this approach is best suited for soft, solid tissues	–	–	–	•	•	–
Glass-bead homogenization	Application of gentle abrasion by vortexing cells with glass beads	•	•	–	–	–	•

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All cell disruption methods cause the release of compartmentalized hydrolases (phosphatases, glycosidases, and proteases) that can alter the protein composition of the lysates. In experiments where relative amounts of protein are to be analyzed, or in experiments involving downstream immunodetection, the data are only meaningful when the protein composition is preserved. Avoid enzymatic degradation by using one or a combination of the following techniques:

- Disrupt the sample or place freshly disrupted samples in solutions containing strong denaturing agents such as 7–9 M urea, 2 M thiourea, or 2% SDS. In this environment, enzymatic activity is often negligible
- Perform cell disruption at low temperatures to diminish enzymatic activity
- Lyse samples at pH >9 using either sodium carbonate or Tris as a buffering agent in the lysis solution (proteases are often least active at basic pH)
- Add a chemical protease inhibitor to the lysis buffer. Examples include phenylmethylsulfonyl fluoride (PMSF), aminoethyl-benzene sulfonyl fluoride (AEBSF), tosyl lysine chloromethyl ketone (TLCK), tosyl phenyl chloromethyl etone (TPCK), ethylenediaminetetraacetic acid (EDTA), benzamidine, and peptide protease inhibitors (for example, leupeptin, pepstatin, aprotinin, and bestatin). For best results, use a combination of inhibitors in a protease inhibitor cocktail
- If protein phosphorylation is to be studied, include phosphatase inhibitors such as fluoride and vanadate

Following cell disruption:

- Check the efficacy of cell wall disruption by light microscopy
- Centrifuge all extracts extensively (20,000 x g for 15 min at 15°C) to remove any insoluble material; solid particles may block the pores of the gel

Protein Solubilization

Protein solubilization is the process of breaking interactions involved in protein aggregation, for example, disulfide bonds, hydrogen bonds, van der Waals forces, ionic interactions, and hydrophobic interactions (Rabilloud 1996). If these interactions are not prevented, proteins can aggregate or precipitate, resulting in artifacts or sample loss. For successful PAGE, proteins must be well solubilized.

Ideally, cell lysis and protein solubilization are carried out in the sample buffer that is recommended for the particular electrophoresis technique, especially when native electrophoresis is the method of choice.

If this is not possible or desirable, proteins must be prepared in sample solubilization solutions that typically contain a number of compounds, including chaotropic agents, detergents, reducing agents, buffers, salts, and ampholytes. These are chosen from a small list of compounds that meet the requirements, both electrically and chemically, for compatibility with the electrophoretic technique being used. In these cases, the sample will have to be diluted with concentrated electrophoresis sample buffer to yield a 1x final buffer concentration.

Detergents

Detergents are classified as nonionic, zwitterionic, anionic, and cationic, and they disrupt hydrophobic interactions between and within proteins (Luche et al. 2003). Some proteins, especially membrane proteins, require detergents for solubilization during isolation and to maintain solubility. Nonionic detergents such as NP-40 and Triton X-100 are not very effective at solubilizing hydrophobic proteins; zwitterionic detergents such as CHAPS and sulfobetaines (for example, SB 3-10 or ASB-14) provide higher solubilization efficiency, especially for integral membrane proteins. Sample preparation for PAGE commonly uses the anionic detergent SDS, which is unparalleled in its ability to efficiently and rapidly solubilize proteins.

Reducing Agents

Thiol reducing agents such as 2-mercaptoethanol (βME) and dithiothreitol (DTT) disrupt intramolecular and intermolecular disulfide bonds and are used to achieve complete protein unfolding and to maintain proteins in their fully reduced states (Figure 3.2).

βME is volatile, evaporates from solution, and reduces protein disulfide bonds by disulfide exchange. There is an equilibrium between free thiols and disulfides, so βME is used in large excess in sample buffers to drive the equilibrium reaction toward completion. If the concentration of βME drops and proteins reoxidize, fuzzy or spurious artifactual bands may result.

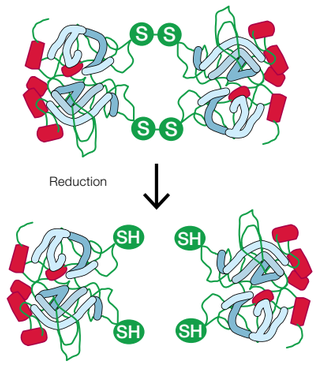

Fig. 3.2. Reduction of proteins with DTT.

DTT is less volatile and is altered during the disulfide reduction reaction to form a ring structure from its original straight chain. The equilibrium favors protein reduction, so lower concentrations of DTT are needed (higher concentrations are recommended for proteins with large numbers of disulfide bonds).

Phosphines such as tributylphosphine (TBP) and Tris-carboxyethylphosphine (TCEP)* offer an alternative to thiols as reducing agents because they can be used at lower concentrations and over a wider pH range than the sulfhydryl reductants.

Chaotropic Agents

Chaotropic compounds such as urea disrupt hydrogen bonds and hydrophobic interactions both between and within proteins. When used at high concentrations, they destroy secondary protein structure and bring proteins into solution that are not otherwise soluble.

Urea and substituted ureas like thiourea improve solubilization of hydrophobic proteins. Currently, the best solution for denaturing electrophoresis is a combination of 7 M urea and 2 M thiourea in combination with appropriate detergents like CHAPS.

Samples containing urea and thiourea can be used in SDS-PAGE when diluted with SDS-PAGE sample buffer. The protein solution should not be heated above 37°C because urea and thiourea get hydrolyzed (to cyanate and thiocyanate, respectively) and modify amino acids on proteins (carbamylation), giving rise to artifactual charge heterogeneity.

Buffers and Salts

Both pH and ionic strength influence protein solubility, making buffer choice important, especially when native electrophoresis conditions are required. Many proteins are more soluble at higher pH; therefore, Tris base is often included to elevate the pH. However, proteins differ in their solubility at different pH values, so different buffers can extract different sets of proteins. The choice of buffer and pH of the sample preparation solution can strongly influence which proteins show up in a separation.

Even in the presence of detergents, some proteins have stringent salt requirements to maintain their solubility, but salt should be present only if it is an absolute requirement. Excess salt in SDS-PAGE samples causes fuzzy bands and narrowing of gel lanes toward the bottom of the gel. If the ionic strength is very high, no bands will appear in the lower part of the gel (a vertical streak will appear instead) and the dye front will be wavy instead of straight. Deionize any sample with a total ionic strength over 50 mM using columns such as Micro Bio-Spin™ Columns, which contain 10 mM Tris at a pH suitable for SDS-PAGE.

*TCEP is included in Bio-Rad's XT Sample Buffers. Although TCEP can be added to SDS-PAGE sample buffer, it must first be neutralized with NaOH; otherwise, it will hydrolyze proteins.

Common Solutions for Protein Solubilization

Ideally, cell lysis and protein solubilization are carried out in the sample buffer that is recommended for the particular electrophoresis technique, especially for native electrophoresis. If this is not possible or desirable, dilute the protein solution with concentrated electrophoresis sample buffer to yield a 1x final buffer concentration.

Formulas for various sample buffers are provided in Part II of this guide.

Removal of Interfering Substances

Success or failure of any protein analysis depends on sample purity. Interfering substances that can negatively impact SDS-PAGE include salts, detergents, denaturants, or organic solvents (Evans et al. 2009). Highly viscous samples indicate high DNA and/or carbohydrate content, which may also interfere with PAGE separations. In addition, solutions at extreme pH values (for example, fractions from ion exchange chromatography) diminish the separation power of most electrophoresis techniques. Use one of the following methods as needed to remove these contaminants:

- Protein precipitation — the most versatile method to selectively separate proteins from other contaminants consists of protein precipitation by trichloroacetic acid (TCA)/acetone followed by resolubilization in electrophoresis sample buffer. A variety of commercial kits can simplify and standardize laboratory procedures for protein isolation from biological samples
- Buffer exchange — size exclusion chromatography is another effective method for removing salts, detergents, and other contaminants

Links

Micro Bio-Spin 6 and Micro Bio-Spin 6 Columns

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Immunoprecipitation

SureBeads™ Protein A and Protein G Magnetic Beads are designed for bioseparation techniques like immunoprecipitation (IP), co-immunoprecipitation (co-IP), and protein pull-down assays (Figure 3.3). SureBeads Beads are superparamagnetic beads with surface activated hydrophilic polymers and are chemically conjugated to Protein A and Protein G to specifically bind to the Fc region of immunoglobulin. This chemistry enables high IgG binding and low nonspecific binding from a variety of biological samples.

Product features include:

- Faster IP — using a magnet beads can be collected faster (within seconds) than with traditional centrifugation-based methods
- Easier IP — ergonomically designed SureBeads magnetic rack magnetizes beads in seconds
- Use less antibody — unique surface chemistry enables proper antibody orientation for optimal antigen binding
- High reproducibility — consistent IgG binding capacity ensures accurate, reproducible results
- Low cost to go magnetic — priced similarly to leading agarose beads

If the sample contains IgG (e.g., tissue lysate, blood-derived sample like plasma/serum) that masks the protein of interest during western blotting of the immunoprecipitated sample, then TidyBlot™ Secondary Reagent is recommended.

For contaminant removal Bio-Rad offers the following (Figure 3.4):

- ReadyPrep™ 2-D Cleanup Kit — uses a modification of the traditional TCA protein precipitation protocol. The kit offers quantitative protein recovery but also ensures easy and reproducible removal of interfering substances
- Bio-Spin® and Micro Bio-Spin 6 Columns — provide rapid salt removal in an easy-to-use spin-column format. Accommodating up to 100 µl of sample, these columns remove compounds <6 kD; proteins can be eluted in electrophoresis sample buffer

Sample Quantitation (Protein Assays)

Determine the concentration of protein in a sample (Berkelman 2008) by using protein assays to:

- Ensure that the amount of protein to be separated is appropriate for the lane dimensions and visualization method
- Facilitate comparison among similar samples; image-based analysis is simplified when equivalent quantities of proteins have been loaded in the lanes of the gel

The most commonly used protein assays are colorimetric assays in which the presence of protein causes a color change that can be measured with a spectrophotometer (Sapan et al. 1999, Noble and Bailey 2009). All protein assays utilize a dilution series of a known protein (usually bovine serum albumin or bovine γ-globulin) to create a standard curve from which the concentration of the sample is derived (for a protocol describing protein quantitation, refer to Part II of this guide).

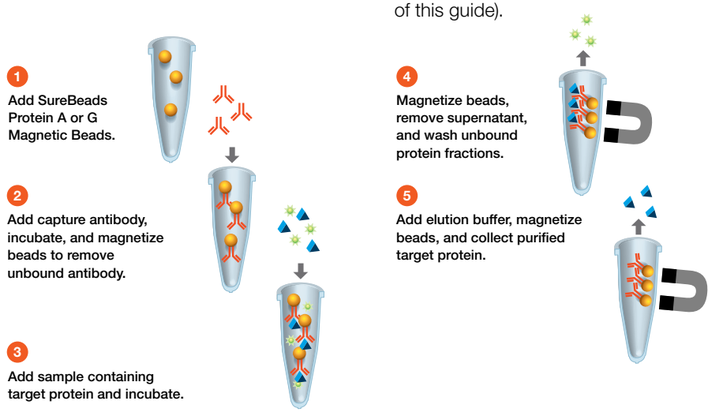

Fig. 3.3. Immunoprecipitation using SureBeads Magnetic Beads.

Links

SureBeads Protein A and Protein B Magnetic Beads

TidyBlot Western Blot Detection Reagent

Bio-Spin 6 and Micro Bio-Spin 6 Columns

ReadyPrep 2-D Cleanup Kit

Table 3.2. Bio-Rad protein assay selection guide.

	Quick Start™ Bradford	Bradford	*DC*™	*RC DC*™
Method				
Bradford	•	•	—	—
Lowry	—	—	•	•
Description	One-step determination; not to be used with high levels of detergents (>0.025% SDS)	Standard Bradford assay, not to be used with elevated levels of detergents (>0.1% SDS)	Detergent compatible *(DC)*; Lowry assay modified to save time	Reducing agent detergent compatible *(RC DC)*
Standard-Concentration Assay				
Sample volume	100 µl	100 µl	100 µl	100 µl
Linear range	0.125–1.5 mg/ml	0.125–1.5 mg/ml	0.125–1.5 mg/ml	0.2–1.5 mg/ml
Low-Concentration Assay				
Sample volume	1 ml	800 µl	200 µl	200 µl
Linear range	1.25–25 µg/ml	1.25–25 µg/ml	5–250 µg/ml	5–250 µg/ml
Microplate assay volume	5 µl	10 µl	5 µl	**
Minimum incubation	5 min	5 min	15 min	15 min
Assay wavelength	595 nm	595 nm	650–750 nm	650–750 nm

Protein Assays

The chemical components of the sample buffer and the amount of protein available for assay dictate the type of assay that may be used (Table 3.2).

- Bradford Assays (Bradford 1976) — are based on an absorbance shift of Coomassie (Brilliant) Blue G-250 Dye under acid conditions. A redder form of the dye is converted into a bluer form upon binding to protein. The increase of absorbance at 595 nm is proportional to the amount of bound dye, and thus to the amount (concentration) of protein in the sample. Compared with other protein assays, the Bradford protein assay is less susceptible to interference by various chemicals that may be present in protein samples*
- Lowry (Lowry et al. 1951) — combines the reactions of cupric ions with peptide bonds under alkaline conditions and the oxidation of aromatic protein residues. The Lowry method is based on the reaction of Cu^{+}, produced by the peptide-mediated reduction of Cu^{2+}, with Folin-Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid in the Folin-Ciocalteu reaction)
- BCA (bicinchoninic acid, Smith et al. 1985) — reacts directly with Cu^{+} (generated by peptide-mediated reduction of Cu^{2+}) to produce a purple end product. The reagent is fairly stable under alkaline conditions and can be included in the copper solution to make the assay a one-step procedure

*The Bradford assay is, however, highly sensitive to ionic detergents like SDS.

To measure protein concentration in Laemmli buffers, use the reducing agent detergent compatible (*RC DC*™) protein assay, which is compatible with reducing agents and detergents. For more information on protein quantitation using colorimetric assays, refer to Bio-Rad bulletin 1069.

Fig. 3.4. Bio-Rad products that can be used for contaminant removal. Top, Micro Bio-Spin and Bio-Spin Columns; Bottom, ReadyPrep 2-D Cleanup Kit.

Related Literature

Modification of Bio-Rad *DC* Protein Assay for Use with Thiols, bulletin 1909

Colorimetric Proteins Assays, bulletin 1069

Links

Sample Buffers and Reagents

Protein Assay Kits and Cuvettes

Disposable Cuvettes for Protein Assays

Quick Start Bradford Protein Assay

Bio-Rad Protein Assay

DC Protein Assay

RC DC Protein Assay

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CHAPTER 4

Reagent Selection and Preparation

This chapter details how to select and prepare the reagents (protein standards, gels, and buffers) required for various PAGE applications. The types of gels and buffers selected should suit the size of the protein under investigation, the desired analysis technique, and the overall goals of the experiment.

Links

Recombinant Protein Standards (Markers)

Precision Plus Protein Unstained Standards

Precision Plus Protein Prestained Standards

Precision Plus Protein All Blue Standards

Precision Plus Protein Dual Color Standards

Precision Plus Protein Dual Xtra Standards

Precision Plus Protein Kaleidoscope Standards

Precision Plus Protein WesternC Standards

Precision Protein StrepTactin-HRP Conjugate

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General Considerations

No particular gel type or buffer is useful for all proteins, and choosing the buffer systems and gel types that offer the highest resolution in the size range of interest may require some experimentation. In selecting reagents for PAGE, consider the following:

- Protein standards — select protein standards that provide maximum resolution in the size range of interest and that offer compatibility and utility for downstream applications such as western blotting
- Gel percentage — choose the percentage that offers the best resolution in the range of interest
- Handcast vs. precast gels — precast gels offer greater convenience and superior quality control and reproducibility than handcast gels; handcast gels provide customized percentages and gradients
- Gel format — select mini- or midi-format gels when throughput is important or sample size is limited; select large-format gels for higher resolution. Select a comb type and gel thickness to accommodate the sample number and volume you are working with
- Buffer system — choose the system that offers the best resolution and compatibility with the protein and application of interest

Protein Standards

Protein standards are mixtures of well-characterized or recombinant proteins that are loaded alongside protein samples in a gel. They are used to monitor separation as well as estimate the size and concentration of the proteins separated in a gel.

Select protein standards that offer:

- Good resolution of the proteins in the size range of interest
- Compatibility with downstream analysis (for example, blotting)

Protein standards are available as prestained or unstained sets of purified or recombinant proteins. In general, prestained standards allow easy and direct visualization of their separation during electrophoresis and their subsequent transfer to membranes. Although prestained standards can be used for size estimation, unstained protein standards will provide the most accurate size determinations.

Applications and details of Bio-Rad's protein standards are provided in Table 4.1.

Recombinant Standards

Recombinant standards are engineered to display specific attributes such as evenly spaced molecular weights or affinity tags for easy detection. Bio-Rad's recombinant standards are the Precision Plus Protein Standards family and are available as stained or unstained standards (Figure 4.1). These standards contain highly purified recombinant proteins with molecular masses of 10–250 kD (or 2–250 kD for the Dual Xtra Standards).

Table 4.1. Applications of Bio-Rad's protein standards.

	Precision Plus Protein™ Standards						Prestained Natural Standards			
	Dual Color	Kaleidoscope™	Dual Xtra	All Blue	WesternC™	Unstained	High Range	Low Range	Broad Range	Natural Kaleidoscope
Electrophoresis										
Accurate MW estimation	•	•	•	•	•	•	–	–	–	–
Visualize electrophoresis	•	•	•	•	•	–	•	•	•	•
Orientation	•	•	•	–	•	–	–	–	–	–
Extended MW range	–	–	•	–	–	–	–	–	–	–
Coomassie staining	•	•	•	•	•	•	•	•	•	•
Fluorescent staining	–	–	–	–	–	•	–	–	–	–
Blotting										
Monitoring transfer efficiency	•	•	•	•	•	–	•	•	•	•
Coomassie staining	•	•	•	•	•	•	•	•	•	•
Immunodetection	–	–	–	–	•	•	–	–	–	–
Fluorescent blots*	•	•	•	•	•	–	–	–	–	–

MW = molecular weight.

* For use with fluorescent blots, not to be confused with fluorescent total blot stains. Precision Plus Protein Prestained Standards contain dyes with fluorescent properties. See bulletin 5723 for details on using precision Plus Protein WesternC Standards for fluorescent multiplexing.

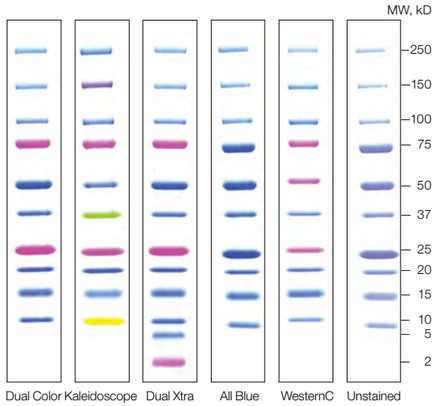

Fig. 4.1. Precision Plus Protein family of protein standards.

- Precision Plus Protein Unstained Standards — include three high-intensity reference bands (25, 50, and 75 kD) and contain a unique affinity *Strep*-tag, which allows detection and molecular weight determination on western blots. These standards offer absolute molecular weight accuracy confirmed by mass spectrometry. Because they contain a known amount of protein in each band, they also allow approximation of protein concentration. These standards are compatible with Laemmli and neutral pH buffer systems and are an excellent choice for use with stain-free technology (since they do not contain dye that can interfere with stain-free detection). See stain-free technology box in Chapter 6 for more details
- Precision Plus Protein Prestained Standards (All Blue, Dual Color, and Kaleidoscope) — include a proprietary staining technology that provides batch-to-batch molecular mass consistency and reproducible migration. The ability to visualize these standards makes them ideal for monitoring protein separation during gel electrophoresis
- Precision Plus Protein Dual Xtra Standards — prestained standards with additional 2 and 5 kD bands to enable molecular mass estimation below 10 kD
- Precision Plus Protein™ WesternC™ Standards — dual color, prestained, and broad range protein standards that enable chemiluminescence detection when probed with StrepTactin-HRP conjugates; the protein standard appears directly on a film or CCD image. Additionally this protein standard has fluorescent properties that enable detection for fluorescent blots*

Polyacrylamide Gels

Polyacrylamide is stable, chemically inert, electrically neutral, hydrophilic, and transparent for optical detection at wavelengths greater than 250 nm. These characteristics make polyacrylamide ideal for protein separations because the matrix does not interact with the solutes and has a low affinity for common protein stains (Garfin 2009).

Polymerization

Polyacrylamide gels are prepared by free radical polymerization of acylamide and a comonomer cross-linker such as bis-acrylamide. Polymerization is initiated by ammonium persulfate (APS) with tetramethylethylenediamine (TEMED) acting as a catalyst (Figure 4.2). Riboflavin (or riboflavin-5'-phosphate) may also be used as a source of free radicals, often in combination with TEMED and APS. Polymerization speed depends on various factors (monomer and catalyst concentration, temperature, and purity of reagents) and must be carefully controlled because it generates heat and may lead to nonuniform pore structures if it is too rapid.

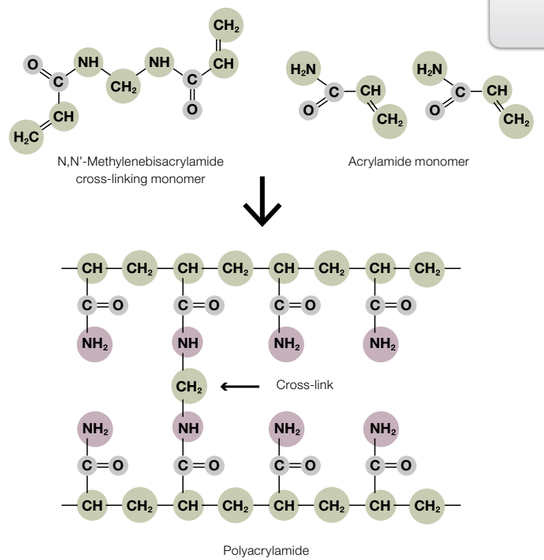

Fig. 4.2. Polymerization of acrylamide monomers and bisacrylamide.

Related Literature

Acrylamide Polymerization — A Practical Approach, bulletin 1156

The Little Book of Standards, bulletin 2414

Protein Standards Application Guide, bulletin 2998

Increase Western Blot Throughput with Multiplex Fluorescent Detection, bulletin 5723

Precision Plus Protein Dual Xtra Standards—New Protein Standards with an Extended Range from 2 to 250 kD, bulletin 5956

Links

Coomassie Stains

Coomassie Brilliant Blue R-250 Stain

Coomassie Brilliant Blue G-250 Stain

Percentage

Polyacrylamide gels are characterized by two parameters: total monomer concentration (%T, in g/100 ml) and weight percentage of cross-linker (%C). By varying these two parameters, the pore size of the gel can be optimized to yield the best separation and resolution for the proteins of interest. %T indicates the relative pore size of the resulting polyacrylamide gel; a higher %T refers to a larger polymer-to-water ratio and smaller average pore sizes.

The practical ranges for monomer concentration are stock solutions of 30–40%, with different ratios of acrylamide monomer to cross-linker. The designations 19:1, 29:1, or 37.5:1 on acrylamide/bis solutions represent cross-linker ratios of 5%, 3.3%, and 2.7% (the most common cross-linker concentrations for protein separations).

$$\%T = \frac{\text{g acrylamide} + \text{g cross-linker}}{\text{Total volume, ml}} \times 100$$

$$\%C = \frac{\text{g cross-linker}}{\text{g acrylamide} + \text{g cross-linker}} \times 100$$

Gels can be made with a single, continuous percentage throughout the gel (single-percentage gels), or they can be cast with a gradient of %T through the gel (gradient gels). Typical gel compositions are between 7.5% and 20% for single-percentage gels, and typical gradients are 4–15% and 10–20%. Use protein migration charts and tables to select the gel type that offers optimum resolution of your sample (Figure 4.3):

- Use single-percentage gels to separate bands that are close in molecular weight. Since optimum separation occurs in the lower half of the gel, choose a percentage in which your protein of interest migrates to the lower half of the gel
- Use gradient gels to separate samples containing a broad range of molecular weights. Gradient gels allow resolution of both high- and low-molecular weight bands on the same gel. The larger pore size toward the top of the gel permits resolution of larger molecules, while pore sizes that decrease toward the bottom of the gel restrict excessive separation of small molecules
- For new or unknown samples, use a broad gradient, such as 4–20% or 8–16%, for a global evaluation of the sample. Then move to using an appropriate single-percentage gel once a particular size range of proteins has been identified

Precast vs. Handcast

Precast gels are ready to use and offer greater convenience, more stringent quality control, and higher reproducibility than handcast gels. Many precast gels also provide a shelf life of up to 12 months, allowing gels to be stored and used as needed (this is not possible with handcast gels, as they degrade within a few days).

Handcast gels, on the other hand, must be prepared from acrylamide and bisacrylamide monomer solutions; the component solutions are prepared, mixed together, and then poured between two glass plates to polymerize (see Part II of this guide for a detailed protocol). Because acrylamide and bisacrylamide are neurotoxins when in solution, care must be taken to avoid direct contact with the solutions and to clean up any spills. In addition, the casting process requires hours to complete, is not as controlled as it is by gel manufacturers, and contributes to more irregularities and less reproducibility with handcast gels.

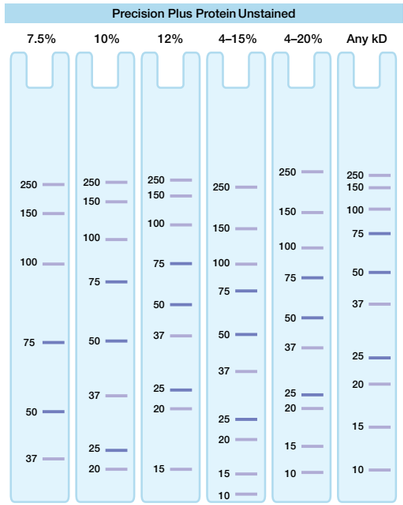

Fig. 4.3. Examples of migration charts.

Although handcasting offers the benefit of customized percentages, chemistries, and gradients, precast gels are sized to fit specific electrophoresis cells and are available in a range of chemistries, formulations, comb types, and thicknesses. Precast gels differ from their handcast counterparts in that they are cast with a single buffer throughout. Bio-Rad's precast gels (Table 4.3) also do not contain SDS and can be used for native or denaturing PAGE. For a complete and current list of available precast gels, visit the Bio-Rad website at **bio-rad.com**.

Format (Size and Comb Type)

The size format of the gel used depends on the electrophoresis cell selected (see Chapter 2). Precast gels are available for Bio-Rad's mini- and midi-format electrophoresis systems, and handcasting accessories are available to fit all Bio-Rad electrophoresis cells.

Additional parameters to consider include the number of wells and gel thickness, which depend on the number and volume of samples to analyze. To create sample wells in a gel, a comb is placed into the top of the gel prior to polymerization. When the comb is removed, a series of sample wells is left behind. The number and size of these wells dictate how many samples and what volume may be loaded (Table 4.2). The thickness of the gel also plays a role in determining the sample volume that can be loaded. A variety of comb types are available for handcasting; refer to **bio-rad.com** for more information.

Table 4.2. Comb types available for Bio-Rad precast polyacrylamide gels.

	Comb Thickness, 1.0 mm	
	Number of Wells	Well Volume
Mini-PROTEAN® Gels (Ready Gel® and Mini-PROTEAN®)	8+1	30 µl
	10	30 µl and 50 µl
	12	20 µl
	15	15 µl
	IPG	7 cm IPG strip
Midi-Format Gels (Criterion™)	12+2	45 µl
	18	30 µl
	26	15 µl
	Prep+2	800 µl
	IPG+1	11 cm IPG strip

Buffer Systems and Gel Chemistries

The pH and ionic composition of the buffer system determine the power requirements and heavily influence the separation characteristics of a polyacrylamide gel.

Buffer systems include the buffers used to:

- Cast the gel
- Prepare the sample (sample buffer)
- Fill the electrode reservoirs (running buffer)

Most common PAGE applications utilize discontinuous buffer systems (Niepmann 2007), where two ions differing in electrophoretic mobility form a moving boundary when a voltage is applied (see Chapter 2). Proteins have an intermediate mobility, making them stack, or concentrate, into a narrow zone at the beginning of electrophoresis. As that zone moves through the gel, the sieving effect of the gel matrix causes proteins of different molecular weights to move at different rates (see Figure 2.2). Varying the types of ions used in the buffers changes the separation characteristics and stability of the gel. Table 4.3 summarizes the various types of gel and buffer systems available.

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Links

Precision Plus Protein Unstained Standards

Broad Range Unstained SDS-PAGE Standards

Links

Mini Format 1-D Electrophoresis Systems

Mini-PROTEAN Precast Gels

Midi Format 1-D Electrophoresis Systems

Criterion Precast Gels

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Table 4.3. Gel and buffer chemistries for PAGE. For a current list of precast gels available from Bio-Rad, visit bio-rad.com.

Gel Type	Selection Criteria	Buffers		Precast (Format) Gels		
		Sample	Running	Mini-PROTEAN*	Criterion	Handcast
SDS-PAGE						
Tris-HCl, pH 8.6	Easy to prepare, reagents inexpensive and readily available; best choice when switching between precast and handcast gels and need to compare results	Laemmli	Tris/glycine/SDS	•	•	•
TGX™	Laemmli-like extended shelf life gels; best choice when long shelf life is needed and traditional Laemmli separation patterns are desired	Laemmli	Tris/glycine/SDS	• (Mini-PROTEAN)	•	—
TGX Stain-Free™	Laemmli-like extended shelf life gels with trihalo compounds for rapid fluorescence detection without staining	Laemmli	Tris/glycine/SDS	• (Mini-PROTEAN)	•	—
Bis-Tris, pH 6.4	Offer longest shelf life, but reagents may be costly	XT	XT MOPS or XT MES	—	•	—
Tris-acetate, pH 7.0	Offer best resolution of high molecular weight proteins; useful in peptide sequencing or mass spectrometry applications	XT or Tricine	XT Tricine or Tris/Tricine/SDS	—	•	•
Native PAGE						
Tris-HCl, pH 8.6	Retention of native protein structure, resolution of proteins with similar molecular weight	Native	Tris/glycine	•	•	•
TGX	Laemmli-like extended shelf life gels; best choice when long shelf life is needed and traditional Laemmli separation patterns are desired	Native	Tris/glycine	• (Mini-PROTEAN)	•	—
Stain-Free	Laemmli-like gels with trihalo compounds for rapid fluorescence detection without staining	Native	Tris/glycine	—	•	—
TGX Stain-Free	Laemmli-like extended shelf life gels with trihalo compounds for rapid fluorescence detection without staining	Native	Tris/glycine	• (Mini-PROTEAN)	•	—
Tris-acetate, pH 7.0	Offer best separation of high molecular weight proteins and protein complexes	Native	Tris/glycine	—	•	•
Peptide Analysis						
Tris-Tricine	Optimized for separating peptide and proteins with molecular weight <1,000	Tricine	Tris/Tricine/SDS	• (Mini-PROTEAN)	•	•
IEF						
IEF	Cast with Bio-Lyte® Ampholytes, allow separation by protein pI; contain no denaturing agents, so IEF is performed under native conditions	50% glycerol	IEF cathode and IEF anode buffers	•	•	—

Laemmli (Tris-HCl)

The Laemmli system has been the standard system for SDS- and native PAGE applications for many years. Many researchers use Tris-HCl gels because the reagents are inexpensive and readily available; precast gels are also readily available in a wide variety of gel percentages.

This discontinuous buffer system relies on the stacking effect of a moving boundary formed between the leading ion (chloride) and the trailing ion (glycinate). Tris buffer is the common cation. Tris-HCl gels can be used in either denaturing SDS-PAGE mode (using Laemmli sample buffer and Tris/glycine/SDS running buffer) or in native PAGE mode (using native sample and running buffers without denaturants or SDS).

Tris-HCl resolving gels are prepared at pH 8.6–8.8. At this basic pH, polyacrylamide slowly hydrolyzes to polyacrylic acid, which can compromise separation. For this reason, Tris-HCl gels have a relatively short shelf life. In addition, the gel pH can rise to pH 9.5 during a run, causing proteins to undergo deamination and alkylation. This may diminish resolution and complicate postelectrophoresis analysis.

Bio-Rad has developed TGX™ (**T**ris-**G**lycine e**X**tended shelf life) Precast Gels, modified Laemmli gels with a proprietary modification that extends shelf life to 12 months and allows gels to be run at higher voltages without producing excess heat. The TGX formulation yields run times as short as 15 min and Laemmli-like separation patterns with exceptionally straight lanes and sharp bands. TGX Gels offer excellent staining quality and transfer efficiency (with transfer times as short as 15 min for tank blotting and as short as 3 min with the Trans-Blot® Turbo™ System), and they do not require special, expensive buffers. Like Tris-HCl gels, TGX Gels use a discontinuous buffer system, with glycinate as the trailing ion, and are, therefore, compatible with conventional Laemmli and Tris/glycine/SDS buffers. These are the best choice when long shelf life is needed and traditional Laemmli separation patterns are desired.

Bio-Rad's TGX Stain-Free™ Gels are Laemmli-like, extended shelf life gels that allow rapid fluorescent detection of proteins with the Gel Doc™ EZ or ChemiDoc™ MP Imaging Systems, eliminating staining/destaining steps for completion of protein separation, visualization, and analysis in 25 min (see stain-free Technology box in Chapter 6 for more details).

Bis-Tris

These systems employ chloride as the leading ion and MES or MOPS as the trailing ion. The common cation is formed from Bis-Tris buffer. The gels are prepared at pH 6.4 to enhance gel stability. Running the same Bis-Tris gels with either MES or MOPS denaturing running buffer produces different migration patterns: MES buffer is used for small proteins, and MOPS buffer is used for mid-sized proteins.

Precast Bis-Tris Gels (for example, Criterion™ XT Bis-Tris Gels) offer extended shelf life (compared to Tris-HCl gels) and room temperature storage. These gels are popular because of their stability but they require special buffers, and the gel patterns cannot be compared to those of Tris-HCl gels.

Common reducing agents such as βME and DTT are not ionized at the relatively low pH of Bis-Tris gels and so do not enter the gel and migrate with the proteins. Alternative reducing agents are, therefore, used with Bis-Tris gels to maintain a reducing environment and prevent protein reoxidation during electrophoresis.

Tris-Acetate

This discontinuous buffer system uses acetate as the leading ion and Tricine as the trailing ion and is ideally suited for SDS-PAGE of large proteins. Tris-acetate gels can be used for both SDS- and native PAGE. Like Bis-Tris gels, they offer extended shelf life and room temperature storage. Because of their lower pH, these gels offer better stability than Tris-HCl gels and are best suited for peptide sequencing and mass spectrometry applications.

Tris-Tricine

One of the drawbacks to using SDS in a separation system is that excess SDS runs as a large front at the low molecular weight end of the separation. Smaller polypeptides do not separate from this front and, therefore, do not resolve into discrete bands. Replacing the glycine in the Laemmli running buffer with Tricine yields a system that separates the small SDS-polypeptides from the broad band of SDS micelles that forms behind the leading-ion front. Proteins as small as 1–5 kD can be separated in these gels.

IEF

Isoelectric focusing (IEF) separates proteins by their net charge rather than molecular weight. IEF gels are cast with ampholytes, amphoteric molecules that generate a pH gradient across the gels. Proteins migrate to their pI, the pH at which the protein has no net charge. Since IEF gels contain no denaturing agents, IEF is performed under native conditions.

Related Literature

Mini-PROTEAN TGX Precast Gels Product Information Sheet, bulletin 5871

Mini-PROTEAN TGX Precast Gel: A Gel for SDS-PAGE with Improved Stability — Comparison with Standard Laemmli Gels, bulletin 5910

Mini-PROTEAN TGX Precast Gel: A Versatile and Robust Laemmli-Like Precast Gel for SDS-PAGE, bulletin 5911

Ready Gel to Mini-PROTEAN TGX Precast Gels Catalog Number Conversion Chart, bulletin 5932

NuPAGE Bis-Tris Precast Gels (MOPS Buffer) to Mini-PROTEAN TGX Precast Gels Catalog Number Conversion Chart, bulletin 5934

Criterion XT Precast Gels Product Information Sheet, bulletin 2911

Criterion TGX Stain-Free Precast Gels Product Information Sheet, bulletin 5974

Links

Mini Format 1-D Electrophoresis Systems

Mini-PROTEAN TGX Precast Gels

Midi Format 1-D Electrophoresis Systems

Criterion TGX Stain-Free Precast Gels

Criterion XT Bis-Tris Precast Gels

Trans-Blot Turbo Transfer System

Imaging Systems

ChemiDoc MP System

Gel Doc EZ Imaging System

Coomassie Stains

Coomassie Brilliant Blue R-250 Stain

Coomassie Brilliant Blue G-250 Stain

Sample Buffers and Reagents

Running Buffers and Reagents

Products for Handcasting Gels

The following products are available to facilitate handcasting gels. For detailed handcasting protocols, refer to Part II of this guide.

Premade Buffers and Reagents

Electrophoresis buffers and reagents are available as individual reagents or as premixed gel-casting, sample, and running buffers. Use of commercially prepared, premixed buffers, which are made with electrophoresis-purity reagents and are quality controlled for reproducible results, saves time but also maximizes reproducibility, prevents potential mistakes in buffer concentration, and standardizes electrophoresis runs. There are no reagents to weigh or filter; just dilute with distilled or deionized water.

AnyGel™ Stands

AnyGel Stands (Figure 4.4) provide stabilization and access to gels for casting and sample loading. The clamping mechanism secures gel cassettes vertically without excess pressure. They are available in two sizes, single- and six-row.

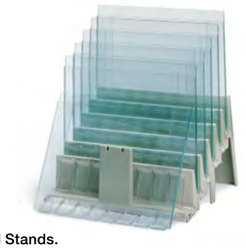

Fig. 4.4. AnyGel Stands.

Multi-Casting Chambers

Multi-casting chambers are used to cast multiple gels of various thicknesses simultaneously. Acrylic blocks act as space fillers when fewer than the maximum number of gels are cast. These chambers work in concert with the gradient formers through a bottom filling port to ensure reproducibility. Multi-casting chambers are available for casting gels for the Mini-PROTEAN, PROTEAN® II, and PROTEAN Plus Systems.

Gradient Formers

Gradient gels have a gradient of acrylamide concentration that increases from top to bottom. To create this gradient, the acrylamide solutions must be mixed in a gradient former before being introduced into the gel cassette. Typically, two solutions are prepared: the light solution (equivalent to the lowest %T in the range to be poured) and a heavy solution (equivalent to the maximum %T to be poured). The most common gradient gel contains 4–20% acrylamide; however, the range of acrylamide concentrations should be chosen on the basis of the size of the proteins being separated.

Two gradient formers are available for PAGE systems. Depending on the gel format, prepare either a single gel using the gradient former or couple the gradient former with a multi-casting chamber to prepare up to 12 gels simultaneously (Figure 4.5).

- Use the Model 485 gradient former to cast a minimum of 4 mini-format gels at a time using the Mini-PROTEAN 3 Multi-Casting Chamber, or to cast a single, large-format (PROTEAN II or PROTEAN Plus) gel
- Use the Model 495 Gradient Former to prepare 4–12 large-format gels (PROTEAN II and PROTEAN Plus) using the multi-gel casting chambers

Fig. 4.5. Multi-casting chambers and gradient formers.

Related Literature

Ready-to-Run Buffers and Solutions Brochure, bulletin 2317

Links

AnyGel Stand

Polyacrylamide Gel Reagents

Premixed Casting Buffers

Mini-PROTEAN Tetra Handcast Systems

PROTEAN Plus Multi-Casting Chamber

PROTEAN Plus Hinged Spacer Plates and Combs

Model 485 Gradient Former

Model 495 Gradient Former

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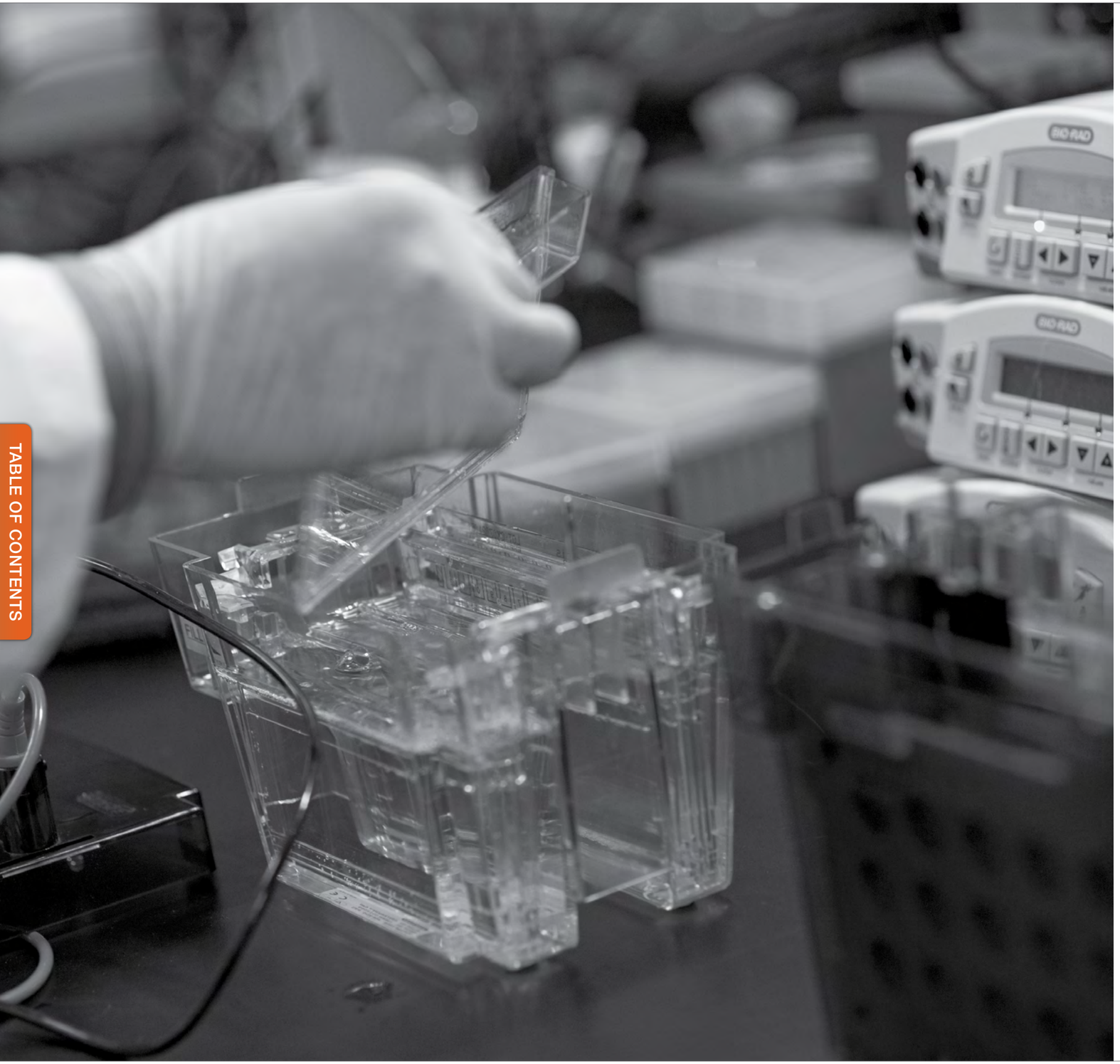

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CHAPTER 5

Performing Electrophoresis

In this phase of the workflow, the electrophoresis system is assembled, samples are loaded, and electrophoresis is initiated by programming the power supply. Select running conditions that provide optimum resolution while maintaining the temperature of the system during separation.

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System Setup

System setup involves placing the gels in the tank, filling the tank with running buffer, loading the samples and protein standards, and programming the power supply. Follow the instructions for system setup in the instruction manuals for the system you are using. General procedures and tips are provided in Part II of this guide.

Running Conditions

Regulated direct current (DC) power supplies allow control over every electrophoresis mode (constant voltage, current, or power). The choice of which electrical parameter to control is usually a matter of preference.

Useful Equations

In PAGE separations, the gel containing the protein sample is placed in the electrophoresis chamber between two electrodes. The driving force behind the separation is the voltage (V, in volts) applied across the electrodes. This leads to a current flow (I, in amperes) through the gel, which has an intrinsic resistance (R, in ohms). Ohm's law describes the mutual dependence of these three parameters:

$$I = V/R \text{ or } V = IR \text{ or } R = V/I$$

The applied voltage and current are determined by the user and the power supply settings; the resistance is inherent in the system and is determined by the ionic strength of the buffer, the conductivity of the gel, and other factors.

The power (P, in watts) consumed by an electrical current element is equal to the product of the voltage and current:

$$P = VI = I^2R = V^2/R$$

The strength of the electric field E (V/cm) applied between the two electrodes is an important parameter in electrophoresis, because it exerts a force on electrically charged objects like proteins and determines their migration rate (where d is the distance in cm):

$$E = V/d$$

Most vertical electrophoresis chambers are operated at a field strength of 10–20 V/cm for 1 mm thick polyacrylamide gels.

Joule Heating

The electric field strength (E, in V/cm) that can be generated between the electrodes is limited by the heat that is inevitably produced during electrophoresis. This Joule heating can lead to band distortion, increased diffusion, and protein denaturation when not efficiently removed from the system. The amount of Joule heating that occurs depends on the conductivity of the buffer used, the magnitude of the applied field, and the total resistance within the system.

The heat generated is proportional to the power consumed (P):

$$\text{Heat} = P/4.18 \text{ cal/sec}$$

Understanding the relationships between power, voltage, current, resistance, and heat is central to understanding the factors that influence the efficiency and efficacy of electrophoresis. The optimum condition is to run at the highest electric field strength possible within the heat dissipation capabilities of the system.

During an electrophoretic separation using the Ornstein-Davis and Laemmli systems, the running buffer warms as a result of Joule heating. The increase in temperature may lead to inconsistent field strength and separation and may cause the buffer to lose its buffering capacity or the gel to melt or become distorted. Under normal running conditions, the running buffer absorbs most of the heat that is generated. However, during extended runs or high-power conditions, active buffer cooling is required to prevent uncontrolled temperature increases.

Other Factors Affecting Electrophoresis

The following variables also change the resistance of the system and, therefore, affect separation efficiency and current and voltage readings:

- Alterations to buffer composition; that is, the addition of SDS or changes in ion concentration due to the addition of acid or base to adjust the pH of a buffer
- Gel pH, ionic strength, and percentage of acrylamide
- Number of gels (current increases as the number of gels increases)
- Volume of buffer (current increases when volume increases)
- Transfer temperature (current increases when temperature increases)
- Gel length (increasing gel length demands higher voltage settings to increase field strength accordingly)
- Gel thickness (increasing gel width or thickness at identical gel length leads to higher current; voltage must be kept unchanged)

Selecting Power Supply Settings

Power supplies that are used for electrophoresis hold one parameter constant (either voltage, current, or power). The PowerPac™ HC and PowerPac Universal Power Supplies also have an automatic crossover capability that allows the power supply to switch over to a variable parameter if a set output limit is reached. This prevents damage to the electrophoresis cell.

The resistance, however, does not remain constant during a run:

- In continuous buffer systems (for example, those used for blotting or DNA separation), resistance declines with increasing temperature caused by Joule heating
- In discontinuous systems, such as the Ornstein-Davis (native) and Laemmli (SDS-PAGE) systems, resistance also changes as discontinuous buffer ion fronts move through the gel; in SDS-PAGE, resistance increases as the run progresses. Depending on the buffer and which electrical parameter is held constant, Joule heating of the gel may increase or decrease over the period of the run

Separations Under Constant Voltage

If the voltage is held constant throughout a separation, the current and power (heat) decrease as the resistance increases. This leads to increased run times, which allow the proteins more time to diffuse. But this appears to be offset by the temperature-dependent increase in diffusion rate of the constant current mode. Separations using constant voltage are often preferred because a single voltage that is independent of the number of gels being run is specified for each gel type.

Separations Under Constant Current

If the current is held constant during a run, the voltage, power, and consequently the heat of the gel chamber increase during the run. As a rule, constant current conditions result in shorter but hotter runs than constant voltage runs.

Separations Under Constant Power

Holding the power constant minimizes the risk of overheating.

General Guidelines for Running Conditions

Electrophoresis cells require different power settings with different buffer systems. The values presented are guidelines — conditions should be optimized for each application. In every case, run the gel until the dye front reaches the bottom of the gel.

Use external cooling during long, unsupervised runs. Temperature-controlled runs often yield more uniform and reproducible results.

For best results:

- Increase run times for gradient gels and decrease them as needed for low molecular weight proteins
- For the first ~10 min of a run, allow the sample to stack using a field strength of 5–10 V/cm gel length. Then continue with the maximum voltage recommended in the instruction manual of the electrophoresis system
- If using multiple cells and constant voltage, use the same voltage for multiple cells as you would for one cell. The current drawn by the power supply doubles with two — compared to one — cells. Set the current limit high enough to permit this additive function. Also be sure to use a power supply that can accommodate this additive current

Gel Disassembly and Storage

Remove the gel cassette and open it according to the manufacturer's instructions. Before handling the gel, wet your gloves with water or buffer to keep the gel from sticking and to minimize the risk of tearing. Sometimes it is also helpful to lift one edge of the gel with a spatula.

Stain, blot, or process the gel as soon as possible to maintain the resolution achieved during electrophoresis and to keep the gel from drying out (see Chapters 6 and 7). For long-term storage, dry stained gels in a 10% glycerol solution (storage at 4°C) between cellophane sheets. This yields clear, publication-quality gels ideal for densitometry.

Links

Power Supplies

PowerPac HC High-Current Power Supply

PowerPac Universal Power Supply

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CHAPTER 6

Protein Detection and Analysis

Following electrophoresis, protein band patterns can be visualized and subjected to qualitative and quantitative analysis. Since most proteins cannot be seen in a gel with the naked eye, protein visualization is usually achieved through use of protein stains. Once the gel is stained, it can be imaged and analyzed using imaging instruments and accompanying software.

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Related Literature

Rapid Validation of Purified Proteins Using Criterion Stain Free Gels, bulletin 6001

Sensitivity and Protein-to-Protein Consistency of Flamingo Fluorescent Gel Stain Compared to Other Fluorescent Stains, bulletin 5705

Comparison of SYPRO Ruby and Flamingo Fluorescent Gel Stains With Respect to Compatibility With Mass Spectrometry, bulletin 5754

Oriole Fluorescent Gel Stain: Characterization and Comparison with SYPRO Ruby Gel Stain, bulletin 5921

Bio-Safe Coomassie Stain Brochure, bulletin 2423

Flamingo Fluorescent Gel Stain Product Information Sheet, bulletin 5346

Links

Criterion Precast Gels

Criterion TGX Stain-Free Precast Gels

Mini-PROTEAN TGX Stain-Free Gels

Coomassie Stains

QC Colloidal Coomassie Stain

Bio-Safe Coomassie Stain

Coomassie Brilliant Blue R-250 Stain

Coomassie Brilliant Blue G-250 Stain

Fluorescent Protein Stains

Flamingo Fluorescent Gel Stain

Oriole Fluorescent Gel Stain

Silver Stains

Negative Stains

Protein Stains

In many cases, the choice of staining technique depends on the availability of imaging equipment. However, a protein staining technique should offer the following features (Miller et al. 2006):

- High sensitivity and reproducibility
- Wide linear dynamic range
- Compatibility with downstream technologies such as protein extraction and assay, blotting, or mass spectrometry
- Robust, fast, and uncomplicated protocol

Staining protocols usually involve the following three steps (protocols are available in Part II of this guide):

- Protein fixation, usually in acidic methanol or ethanol (a few staining protocols already contain acid or alcohols for protein fixation and so do not require this separate step)
- Exposure to dye solution
- Washing to remove excess dye (destaining)

Total Protein Stains

Total protein stains allow visualization of the protein separation pattern in the gel. Table 6.1 compares the advantages and disadvantages of several total protein staining techniques.

- Coomassie stains — the most popular anionic protein dye, Coomassie (Brilliant) Blue stains almost all proteins with good quantitative linearity at medium sensitivity. There are two variants of Coomassie (Brilliant) Blue: R-250 (R for reddish), which offers shorter staining times, and G-250 (G for greenish), which is available in more sensitive and environmentally friendly formulations (Simpson 2010, Neuhoff et al. 1988). Coomassie dyes are also the favorite stains for mass spectrometry and protein identification. Bio-Rad offers three variations of Coomassie Blue Stains. The QC Colloidal Coomassie Stain provides sensitivity down to ~3 ng BSA, low background endpoint staining, and reproducibility that is needed in regulated environments. Additionally, this colloidal stain is formulated to be ready-to-use (no additional prep or dilution needed) and to be environmentally friendly (no methanol or acetic acid waste disposal). Bio-Safe™ Coomassie Stain is a nonhazardous formulation of Coomassie Blue G-250 that requires only water for rinsing and destaining. It offers a sensitivity that is better than conventional Coomassie R-250 formulations and equivalent to Coomassie Blue G-250, but with a simpler and quicker staining protocol
- Fluorescent stains — fulfill almost all of the requirements for an ideal protein stain by offering high sensitivity, a wide linear dynamic range over four orders of magnitude, a simple and robust protocol, and compatibility with mass spectrometry. In comparison to Coomassie or silver staining techniques, however, fluorescent dyes are more expensive and require either a CCD (charge-coupled device) camera or fluorescence scanner for gel imaging. For these reasons, fluorescent stains are often used in proteomics applications and on 2-D gels, where the relative quantitation of proteins in complex mixtures is performed over several orders of abundance and protein identity is determined using in-gel proteolytic digestion and mass spectrometry. Examples include Flamingo™ and Oriole™ Fluorescent Gel Stains
- Silver stains — offer the highest sensitivity, but with a low linear dynamic range (Merril et al. 1981, Rabilloud et al. 1994). Often, these protocols are time-consuming, complex, and do not offer sufficient reproducibility for quantitative analysis. In addition, their compatibility with mass spectrometry for protein identification purposes is lower than that of Coomassie stains and fluorescent dyes (Yan et al. 2000)
- Negative stains — rapid negative stains require only ~15 min for high-sensitivity staining, where protein bands appear as clear areas in a white background. Zinc and copper stains do not require gel fixation, ensuring that proteins are not altered or denatured. Negative stains can be used as a quality check before transfer to a western blot or analysis by mass spectrometry, although they are not the best choice when quantitative information is desired
- Stain-free technology — a haloalkane compound in Bio-Rad's Criterion™, Criterion™ TGX, and Mini-PROTEAN® TGX Stain-Free™ Gels covalently binds to tryptophan residues of proteins when activated with UV light. This allows protein detection (with a stain-free compatible imager) in a gel both before and after transfer, as well as total protein detection on a blot when using PVDF membranes (see stain-free technology box)

Specific Protein Stains

Specific protein stains are used to visualize specific protein classes such as glycoproteins (Hart et al. 2003) and phosphoproteins (Steinberg et al. 2003, Agrawal and Thelen 2009), which are of special interest to researchers working in the life sciences (examples include Pro-Q Diamond and Pro-Q Emerald).

Table 6.1. Bio-Rad Gel stain selection guide.

Total Protein Stain	Sensitivity (Lower Limit)	Time	Comments	Detection Method
Stain-Free Imaging				**Fluorescence**
Stain-Free	2–28 ng	<5 min	Rapid; compatible with blotting and mass spectrometry; simple protocol that does not require additional reagents; requires tryptophan residues in protein	Uses stain-free compatible imaging system for detection
Coomassie Stains				**Colorimetric**
QC Colloidal Coomassie	3 ng	1–20 hr	Colloidal endpoint stain; premixed; nonhazardous formulation	
Bio-Safe Coomassie G-250	8–28 ng	1–2.5 hr	Nonhazardous staining in aqueous solution; premixed, mass spectrometry compatible	
Coomassie Brilliant Blue R-250	36–47 ng	2.5 hr	Simple and consistent; mass spectrometry compatible; requires destaining with methanol	
Silver Stains				**Colorimetric**
Dodeca™ Silver Stain Kit	0.25–0.5 ng	3 hr	Simple, robust; mass spectrometry compatible; ideal for use with Dodeca stainers (Sinha et al. 2001)	
Silver Stain Plus™ Kit	0.6–1.2 ng	1.5 hr	Simple, robust; mass spectrometry compatible (Gottlieb and Chavko 1987)	
Silver Stain (Merril et al. 1981)	0.6–1.2 ng	2 hr	Stains glycoproteins, lipoproteins, lipopolysaccharides, nucleic acids	
Fluorescent Stains				**Fluorescent**
Oriole Fluorescent Gel Stain*	0.5–1 ng	1.5 hr	Rapid protocol, requires no destaining, mass spectrometry compatible; compatible only with UV excitation	
Flamingo Fluorescent Gel Stain	0.25–0.5 ng	5 hr	High sensitivity; broad dynamic range; simple protocol requires no destaining; mass spectrometry compatible; excellent for laser-based scanners	
SYPRO Ruby Protein Gel Stain	1–10 ng	3 hr	Fluorescent protein stain; simple, robust protocol;broad dynamic range; mass spectrometry compatible	
Negative Stains				**Colorimetric**
Zinc Stain	6–12 ng	15 min	High-contrast results; simple, fast, and reversible; compatible with elution or blotting as well as mass spectrometry (Fernandez-Patron et al. 1992)	
Copper Stain	6–12 ng	10 min	Single reagent; simple, fast, and reversible; compatible with elution or blotting as well as mass spectrometry (Lee et al. 1987)	

* Do not use Oriole Gel Stain with native gels.

Stain-Free Technology

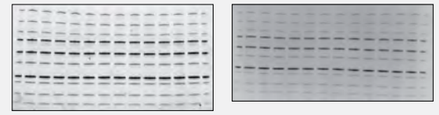
Bio-Rad's stain-free technology allows direct visualization, analysis, and documentation of protein samples in PAGE gels without staining, destaining, or gel drying.

The system comprises the Gel Doc™ EZ and ChemiDoc™ MP Imagers, Image Lab™ Software, and precast gels that include unique trihalo compounds that allow rapid fluorescent detection of proteins — without staining. The trihalo compounds react with tryptophan residues in a UV-induced reaction to produce fluorescence, which can be detected by the imager either within gels or on low-fluorescence PVDF membranes. Activation of the trihalo compounds in the gels adds 58 Da moieties to available tryptophan residues and is required for protein visualization. Proteins that do not contain tryptophan residues are not detected.

The sensitivity of the stain-free system is comparable to staining with Coomassie (Brilliant) Blue for proteins with a tryptophan content >1.5%; sensitivity superior to Coomassie staining is possible for proteins with a tryptophan content >3%.

Benefits of stain-free technology include:

- Elimination of staining and destaining steps for faster results
- Automated gel imaging and analysis
- No background variability within a gel or between gels (as is often seen with standard Coomassie staining)
- Visualization of transferred (blotted) proteins on low-fluorescence PVDF membranes
- Reduced organic waste by eliminating the use of acetic acid and methanol in staining and destaining



Gel (left) and blot imaged using stain-free technology.

Links

SYPRO Ruby Protein Gel Stain

Dodeca Silver Stain Kit

Silver Stain Plus Kit

Zinc Stain

Copper Stain

Imaging Systems

ChemiDoc MP System

Gel Doc EZ System

Image Lab Software

Related Literature

Bio-Rad Imaging Systems Family Brochure, bulletin 5888

Imaging Fluorescently Stained Gels with Image Lab Software Quick Start Guide, bulletin 5989

Links

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Dodeca™ High-Throughput Stainers

Dodeca stainers are high-throughput gel staining devices available in two sizes (Figure 6.1): the small size accommodates up to 24 Criterion Gels while the large size can accommodate up to 12 large-format gels. The stainers ensure high-quality, consistent results and eliminate gel breakage from excess handling.

The stainers feature a shaking rack that holds staining trays at an angle to allow air bubbles to escape and ensure uniform gel staining by protecting gels from breaking. They are compatible with the following stains:

- Bio-Safe Coomassie (Brilliant) Blue G-250 Stain
- Coomassie (Brilliant) Blue R-250 Stain
- Flamingo Fluorescent Gel Stain
- SYPRO Ruby Protein Gel Stain
- Oriole Fluorescent Gel Stain
- Dodeca Silver Stain Kits

Imaging

Though total protein stains yield visible band patterns, in modern laboratory environments, electrophoresis patterns (called electropherograms) are digitized by dedicated image acquisition devices and data are analyzed with sophisticated software. Once the gels are digitized, the raw data can be stored for further reference.

Imaging Systems

Selecting image acquisition devices for the digitization of electrophoresis gels depends on the staining technique used (see also Table 6.2):

- Flat-bed densitometers — based on high-performance document scanners that have been modified to make them suitable for accurate scientific measurement of optical density. Modifications include automatic calibration to traceable reference standards, mathematical correction of image non-uniformity, and environmental sealing against liquid spills in the laboratory. Densitometers measure the absorbance (for gels stained with visible dyes) or reflectance (of blots developed with colorimetric reagents) of visible light. Bio-Rad's GS-900™ Calibrated Densitometer provides a claibrated linear dynamic range to a NIST-traceable standard up to 3.4 optical density (OD) units
- CCD (charge-coupled device) cameras — operate with either trans-illumination provided by light boxes (visible or UV) positioned underneath the gel or blot for imaging a variety of stains (Coomassie, silver, fluorescence) or epi-illumination detected using colorimetric or fluorescent techniques. Supercooled CCD cameras reduce image noise, allowing detection of faint luminescent signals. Bio-Rad's Gel Doc™ EZ System provides four application-specific trays: a UV tray (for ethidium bromide staining of DNA gels and fluorescence imaging), a white tray (for Coomassie, copper, silver, and zinc stains), a blue tray (for nondestructive nucleic acid imaging), and a stain-free tray for direct visualization, analysis, and documentation of protein samples in polyacrylamide gels without staining, destaining, or gel drying (see Stain-Free technology box)

Fig. 6.1. High-throughput Dodeca Gel Stainers.

Table 6.2. Bio-Rad imaging system selection guide.

Application	ChemiDoc™ MP	ChemiDoc	ChemiDoc XRS+	Gel Doc™ XR+	GS-900™	Gel Doc EZ
Blot Detection						
Multiplex fluorescence	✓	–	–	–	–	–
Chemiluminescence	✓	✓	✓	–	–	–
Stain-free blots	✓	✓	✓	✓	–	✓
Colorimetric	✓	✓	✓	–	–	–
SYPRO Ruby Protein Blot Stain*	✓	✓	✓	–	–	–
Nucleic Acid Detection						
Ethidium bromide stain	✓	✓	✓	✓	–	✓
SYBR® Green I and SYBR® Safe Stains	✓	✓	✓	✓	–	✓
Fast Blast™ DNA Stain	✓	✓	✓	✓	✓	✓
Protein Detection, 1-D Gels						
Stain-free gels	✓	✓	✓	✓	–	✓
Coomassie blue stain	✓	✓	✓	✓	✓	✓
Silver stain	✓	✓	✓	✓	✓	✓
SYPRO Ruby Protein Gel Stain and Flamingo™ and Oriole™ Fluorescent Gel Stains	✓	✓	✓	✓	–	✓
Protein Detection, 2-D Gels						
Coomassie blue stain	✓	✓	✓	✓	✓	✓
Silver stain	✓	✓	✓	✓	✓	✓
SYPRO Ruby Protein Gel Stain and Flamingo and Oriole Fluorescent Gel Stains	✓	✓	✓	✓	–	✓
Pro-Q Stain	✓	✓	✓	✓	–	✓
Cy2, Cy3, Cy5 Label	✓	–	–	–	–	–

✓ Recommended; — not recommended.
* Optimal with low fluorescence PVDF membrane.

Imaging Software

A robust software package is required for image acquisition to analyze data and draw conclusions from PAGE applications. Sophisticated gel analysis software provides a variety of tools that enhance the user's ability to evaluate the acquired data. The software adjusts contrast and brightness, magnifies, rotates, resizes, and annotates gel images, which can then be printed using standard and thermal printers. All data in the images can be quickly and accurately quantified. The software can measure total and average quantities and determine relative and actual amounts of protein. Gel imaging software is also capable of determining the presence/absence and up/down regulation of proteins, their molecular weight, pI, and other values. For more information on imagers and gel evaluation software, visit bio-rad.com.

Bio-Rad offers three different software packages for gel imaging and analysis:

- Image Lab™ Software — image acquisition and analysis software that runs the ChemiDoc MP, Gel Doc XR+, Gel Doc EZ, and ChemiDoc XRS+ Imaging Systems. The software allows automatic configuration of these imaging systems with appropriate filters and illumination sources. It also allows manual or automated analysis of PAGE gels and western blots
- Quantity One® 1-D Analysis Software — acquires, quantitates, and analyzes a variety of data, including radioactive, chemiluminescent, fluorescent, and color-stained samples acquired from densitometers, storage phosphor imagers, fluorescence imagers, and gel documentation systems. The software allows automatic configuration of these imaging systems with appropriate filters, lasers, LEDs, and other illumination sources. It also allows manual or automated analysis of PAGE gels and western blots
- PDQuest™ 2-D Analysis Software — used for 2-D gel electrophoretic analysis

Links

ChemiDoc MP System
ChemiDoc XRS+ System

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Related Literature

Molecular Weight Determination by SDS-PAGE, bulletin 3133

Using Precision Plus Protein Standards to Determine Molecular Weight, bulletin 3144

Molecular Weight Estimation Using Precision Plus Protein WesternC Standards on Criterion Tris-HCl and Criterion XT Bis-Tris Gels, bulletin 5763

Molecular Weight Estimation and Quantitation of Protein Samples Using Precision Plus Protein WesternC Standards, the Immun-Star WesternC Chemiluminescent Detection Kit, and the Molecular Imager ChemiDoc XRS Imaging System, bulletin 5576

Analysis

Beyond protein band patterns, PAGE can yield information about a protein's size (molecular weight) and yield (quantity). Image analysis software greatly enhances and facilitates these measurements.

Molecular Weight (Size) Estimation

SDS-PAGE is a reliable method for estimating the molecular weight (MW) of an unknown protein, since the migration rate of a protein coated with SDS is inversely proportional to the logarithm of its MW. The key to accurate MW determination is selecting separation conditions that produce a linear relationship between log MW and migration within the likely MW range of the unknown protein. A protocol for MW estimation is provided in Part II of this guide.

To ensure accurate MW determination:

- Separate the protein sample on the same gel with a set of MW standards (see Chapter 3 for information regarding selection of protein standards)
- For statistical significance, generate multiple data points (>3 lanes per sample)
- Use a sample buffer containing reducing agents (DTT or βME) to break disulfide bonds and minimize the effect of secondary structure on migration
- Include SDS in the sample buffer. SDS denatures secondary, tertiary, and quaternary structures by binding to hydrophobic protein regions. SDS also confers a net negative charge on the proteins, which also results in a constant charge-to-mass ratio

After separation, determine the relative migration distance (R_f) of the protein standards and of the unknown protein. R_f is defined as the mobility of a protein divided by the mobility of the ion front. Because the ion front can be difficult to locate, mobilities are normalized to the tracking dye that migrates only slightly behind the ion front:

$$R_f = \text{(distance to band)/(distance to dye front)}$$

Using the values obtained for the protein standards, plot a graph of log MW vs. R_f (Figure 6.3). The plot should be linear for most proteins, provided that they are fully denatured and that the gel percentage is appropriate for the MW range of the sample. The standard curve is sigmoid at extreme MW values because at high MW, the sieving affect of the matrix is so large that molecules are unable to penetrate the gel. At low MW, the sieving effect is negligible and proteins migrate almost freely. To determine the MW of the unknown protein band, interpolate the value from this graph.

The accuracy of MW estimation by SDS-PAGE is in the range of 5–10%. Glyco- and lipoproteins are usually not fully coated with SDS and will not behave as expected in SDS-PAGE, leading to false estimations. For more details about molecular weight estimation using SDS-PAGE, refer to bulletin 3133.

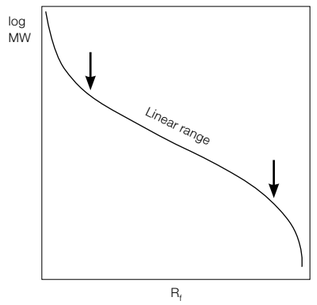

Fig. 6.3. Typical characteristics of a log MW vs. R_f curve for protein standards.

Quantitation

Of all the methods available for protein quantitation (including UV spectroscopy at 280 nm, colorimetric dye-based assays, and electrophoresis in combination with image acquisition analysis), only protein quantitation by electrophoresis enables evaluation of purity, yield, or percent recovery of individual proteins in complex sample mixtures.

Two types of quantitation are possible: relative quantitation (quantitation of one protein species relative to the quantity of another) and absolute quantitation (quantitation of a protein by using a calibration curve generated by a range of known concentrations of that protein). Because proteins interact differently with protein stains, the staining intensity of different proteins at identical protein loads can be very different. Thus, only relative quantitative values can be determined in most cases. Absolute protein measurements can only be made if the protein under investigation is available in pure form and is used as the calibrant.

For protein quantitation using PAGE to be of value:

- Employ sample preparation procedures that avoid nonspecific protein loss due to insolubility, precipitation, and absorption to surfaces
- Ensure all proteins enter the electrophoretic separation medium
- Optimize the quality of the electrophoretic separation. For example, wavy, distorted protein bands and comigration of bands lead to questionable results
- When possible, separate a dilution series of pure proteins in parallel. This enables the creation of a calibration curve (as for molecular weight determination with SDS-PAGE, above)
- Analyze all samples (including samples for calibration) at least in duplicate
- Use a stain that offers sufficient sensitivity and a high dynamic range. Fluorescent stains like Flamingo and Oriole Fluorescent Gel Stains are recommended over Coomassie and silver staining techniques

Total Protein Normalization

Western blotting is a widely used method for quantifying protein expression. Changes in expression levels are identified by comparing band intensities between different samples or different experimental conditions. In order to correct for variations in sample preparation, sample loading, and/or transfer efficiency researchers need to normalize signal of interest (band) intensity against a reference. This reference should vary only proportionally with the amount of sample loaded. Highly expressed housekeeping proteins, such as actin, ß-tubulin, or GAPDH, are often assumed to be stable reference proteins and are often used in normalization.

Stain-free technology allows normalization by measuring total protein directly in the gel or on the membrane that is used for western blotting. This eliminates the need to cut, strip, and reprobe blots required for housekeeping protein normalization strategies and thus saves time and improves the precision and reliability of western blotting data. Total protein normalization using stain-free technology has a broader dynamic range (Figure 6.4) and is more effective at detecting small-fold changes in protein expression and regulation than normalization using housekeeping proteins.

Bio-Rad provides imaging systems, software, and gels for total protein normalization:

- ChemiDoc Imaging Systems – stain-free enabled imaging systems available for chemiluminescence and fluorescence imaging
- Image Lab Software – intuitive software that facilitates easy total protein normalization and protein quantitation using ChemiDoc Imaging Systems
- Precast and handcast stain-free SDS-PAGE gels – the unique chemistry of Criterion and Mini-PROTEAN TGX Stain-Free gels allows rapid fluorescent detection of total protein

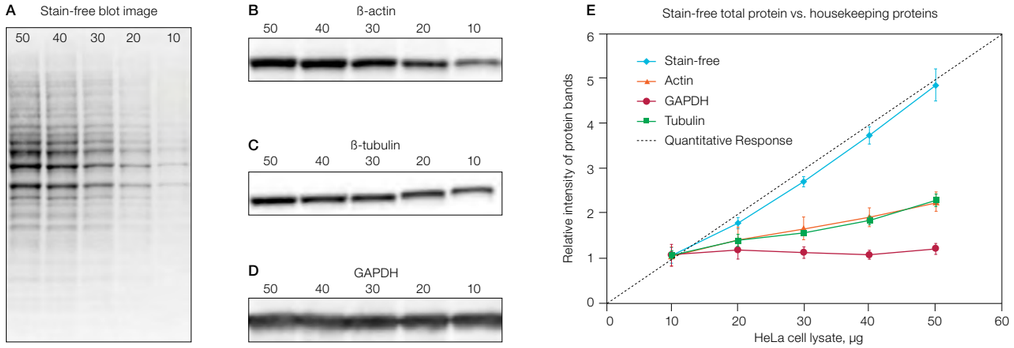

Fig. 6.4. Comparison of protein normalization using stain-free technology and commonly used housekeeping proteins. Tenfold dilutions of HeLa cell lysates ranging from 50 to 10 µg were loaded for samples detected with stain-free technology **(A)** and the housekeeping genes β-actin **(B)**, β-tubulin **(C)**, and GAPDH **(D)**. The protein quantification signal is higher with stain-free technology than with housekeeping genes **(E)**.

Links

Image Lab Software
Imaging Systems
Gel Doc XR+ System
Gel Doc EZ System
ChemiDoc MP System
ChemiDoc XRS+ System
Quantity One 1-D Analysis Software
PDQuest 2-D Analysis Software

Links

Fluorescent Protein Stains
Flamingo Fluorescent Gel Stain
Oriole Fluorescent Gel Stain
Coomassie Stains
Silver Stains

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CHAPTER 7

Downstream Applications

Following electrophoresis, the entire gel might be blotted (proteins transferred to a membrane) or dried, or individual proteins might be excised or eluted from the gel for analysis.

Western Blotting (Immunoblotting)

When specific antibodies are available, transferring the proteins to a membrane (blotting) followed by immunological staining is an attractive complement to general protein stains and provides additional information. A typical immunoblotting experiment consists of five steps (Figure 7.1). Following PAGE:

1. Proteins are transferred from the gel to a membrane where they become immobilized as a replica of the gel's band pattern (blotting).
2. Unoccupied protein-binding sites on the membrane are saturated to prevent nonspecific binding of antibodies (blocking).
3. The blot is probed for the proteins of interest with specific primary antibodies.
4. Secondary antibodies, specific for the primary antibody type and conjugated to detectable reporter groups such as enzymes or radioactive isotopes, are used to label the primary antibodies.
5. Labeled protein bands are visualized by the bound reporter groups acting on an added substrate or by radioactive decay.

Bio-Rad offers a complete range of products for blotting, including blotting cells for protein transfers, blotting membranes, filter paper, premixed blotting buffers, reagents, protein standards, and detection kits. Please refer to the Protein Blotting Guide (Bio-Rad bulletin 2895) for more information.

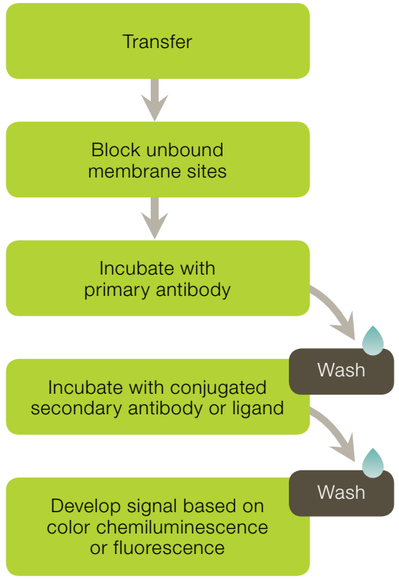

Fig. 7.1. Western blotting workflow.

Immunodetection

PrecisionAb™ Validated Antibodies for Western Blotting

The PrecisionAb Antibody portfolio is a premium collection of highly specific and sensitive primary antibodies that have been extensively validated for western blotting for consistent performance with minimal need for optimization. All antibodies are tested using whole cell or tissue lysates expressing endogenous levels of the target proteins (no overexpression by transfection or target enrichment). A detailed protocol and complete western blot image is provided so that the data can easily be replicated with complete confidence. Trial sizes of antibodies with positive control lysates allow easy access for testing performance before buying larger quantities.

Bulk quantities of these antibodies can be ordered by contacting the antibody specialists.

Immun-Star™ AP & HRP Secondary Antibody Conjugates

Bio-Rad's Immun-Star range offers a suite of affinity-purified (high purity), cross-adsorbed (high specificity), blotting-grade HRP- and AP-conjugated goat anti-mouse and goat anti-rabbit secondary antibodies for easy and sensitive colorimetric or chemiluminescent western blot detection. High titer of the blotting-grade antibody conjugates increases assay sensitivity. High titer also allows greater working dilutions, decreasing background and increasing the signal-to-noise ratio. An ensemble of related product offerings includes AP substrate, substrate packs, and complete detection kits.

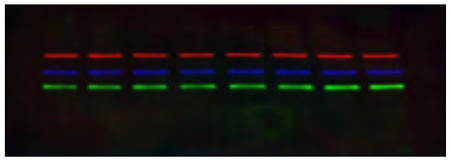

Fig. 7.2. Triplex western blot imaged by the ChemiDoc MP Imaging System.
Target protein #1 (ATG7): Red — StarBright™ B700*
Target protein #2 (AKR1C2): Green — DyLight 800
Normalization protein (tubulin): Blue — hFAB™ Rhodamine*

* Fluorescent labeled antibodies exclusive to Bio-Rad Laboratories.

Fluorescent secondary antibodies for multiplex western blotting

StarBright Blue 700 Secondary Antibodies (Goat Anti-Mouse and Goat Anti-Rabbit) — Unmatched Sensitivity and Easy Multiplexing

StarBright Blue 700 (Ex/Em = 470 nm/700 nm) is a new ultra-sensitive fluorescent label that allows detection of low abundance proteins in seconds of exposure time with minimal background. Highly cross-adsorbed secondary antibodies conjugated to StarBright are ideal for fluorescent western blotting — either for the detection of a single target protein or for multiplex detection of several proteins on one blot, without stripping and reprobing. The StarBright Fluorophore is composed of a condensed polymer made up of multiple light-absorbing and -emitting monomers, which provides an exceptionally bright signal compared to most traditional fluorophores.

StarBright Blue 700 Fluorescent Secondary Antibodies can be used with traditional fluorophores like RGB fluorophores and IR 800 dyes for multiplexing. In addition, StarBright Antibodies can be used with Bio-Rad's stain-free technology and/or hFAB Rhodamine Anti-Housekeeping Primary Antibodies for protein normalization. These antibodies are optimized for use with the ChemiDoc™ MP Imaging System, permitting detection of multiple proteins in a single blot. This can save time, sample, and reagents.

hFAB Anti-Housekeeping antibodies (Anti-Actin, Anti-Tubulin, and Anti-GAPDH) — never worry about cross-reactivity

hFAB Anti-Housekeeping Protein Antibodies are human Fab fragments directly labeled with rhodamine (Ex/Em = 530 nm/570 nm). These antibodies allow easy, one-step detection of common housekeeping proteins like actin, tubulin, and GAPDH in human, mouse, and rat samples without the need for a secondary antibody. These antibodies are created using Bio-Rad's Human Combinatorial Antibody Library (HuCAL®) technology. This ensures no species cross-reactivity, which means they can be used in multiplex western blots with primary antibodies from any host species.

Electroelution

Electroelution, as its name implies, is a technique that applies the principles of electrophoresis to enable recovery (elution) of molecules such as proteins from gels and gel slices. It can be used with either slices from a gel containing the protein of interest or with entire preparative gels. Electroelution uses an electrical field and the charged nature of proteins to move them from the gel and into a buffer solution. Once eluted, proteins can be assayed for activity, applied to subsequent purification steps, or subjected to mass spectrometry or a variety of other applications.

The Model 422 Electro-Eluter (Figure 7.3) combines with the tank and lid of the Mini Trans-Blot® Cell (or older Mini-PROTEAN® II or Mini-PROTEAN 3 Cells) to elute macromolecules from single or multiple gel slices. The electro-eluter has six vertical glass tubes connecting the upper and lower buffer chambers. A frit at the bottom of each tube retains the gel slice but permits macromolecules to migrate through when current is applied. When the macromolecules have passed through the frit, they are collected in the membrane cap for further analysis or testing. Depending on the buffer system, the Model 422 Electro-Eluter can be used for elution or dialysis of up to six samples.

Fig. 7.3. Model 422 Electro-Eluter

Related Literature

Protein Blotting Guide, A Guide to Transfer and Detection, bulletin 2895

Western Blotting Detection Reagents Brochure, bulletin 2032

Links

Model 422 Electro-Eluter
Mini Trans-Blot Cell
Mini-PROTEAN Cell
PrecisionAb Antibodies
Immun-Star HRP and AP Conjugates
StarBright Secondary Antibodies

Links

hFAB Anti-Housekeeping Antibodies
Silver Stains
Coomassie Stains
Fluorescent Protein Stains
Flamingo Fluorescent Gel Stain
Oriole Fluorescent Gel Stain
SYPRO Ruby Protein Gel Stain

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Protocols

Sample Preparation

General Tips for Sample Preparation

Keep the sample preparation workflow simple (increasing the number of sample handling steps may increase variability).

Lysis (Cell Disruption)

- Suspend ~1 mg (wet weight) pelleted cells in ~10 µl SDS-PAGE sample buffer for a protein concentration of 3–5 µg/µl. If disrupted in liquid nitrogen, tissue samples like liver biopsies and plant leaves contain 10–20% and 1–2% protein, respectively
- To diminish endogenous enzymatic activity:
  - Disrupt the sample or place freshly disrupted samples in solutions containing strong denaturing agents such as 7–9 M urea, 2 M thiourea, or 2% SDS. In this environment, enzymatic activity is often negligible
  - Perform cell disruption at low temperatures to diminish enzymatic activity
  - Lyse samples at pH >9 using either sodium carbonate or Tris as a buffering agent in the lysis solution (proteases are often least active at basic pH)
  - Add a chemical protease inhibitor to the lysis buffer. Examples include phenylmethylsulfonyl fluoride (PMSF), aminoethyl-benzene sulfonyl fluoride (AEBSF), tosyl lysine chloromethyl ketone (TLCK), tosylphenylchloromethyletone (TPCK), ethylenediaminetetraacetic acid (EDTA), benzamidine, and peptide protease inhibitors (for example, leupeptin, pepstatin, aprotinin, and bestatin). For best results, use a combination of inhibitors in a protease inhibitor cocktail
  - If protein phosphorylation is to be studied, include phosphatase inhibitors such as fluoride and vanadate
- When working with a new sample, use at least two different cell disruption protocols and compare the protein yield (by protein assay) and qualitative protein content (by SDS-PAGE)
- Optimize the power settings of mechanical rupture systems and incubation times for all lysis approaches. Because mechanical cell lysis usually generates heat, employ cooling where required to avoid overheating of the sample
- Following cell disruption, check the efficacy of cell wall disruption by light microscopy and centrifuge all extracts extensively (20,000 x g for 15 min at 15°C) to remove any insoluble material; solid particles may block the pores of the electrophoresis gel

Protein Solubilization

- Solubilize proteins in a buffer that is compatible with the corresponding electrophoresis technique
- Dissolve pelleted protein samples in 1x sample buffer
- Dilute dissolved protein samples with sample buffer stock solutions to a final sample buffer concentration of 1x
- Perform a protein quantitation assay to determine the amount of total protein in each sample. Use a protein assay that is tolerant to chemicals in your samples. For samples in Laemmli sample buffer, for example, use the *DC*™ or *RC DC*™ Protein Assays, which can tolerate up to 10% detergent. Omit the protein assay if sample amount is limited.
- Dilute or concentrate samples as needed to yield a final protein concentration of >0.5 mg/ml
- Use protein extracts immediately or aliquot them into appropriately sized batches and store them at –80°C to avoid freeze-thaw cycles
- For long-term sample storage, store aliquots at –80°C; avoid repeated thawing and freezing of protein samples
- Highly viscous samples likely have a very high DNA or carbohydrate content. Fragment DNA with ultrasonic waves during protein solubilization or by adding endonucleases like benzonase. Use protein precipitation with TCA/acetone (for example, with the ReadyPrep™ 2-D Cleanup Kit) to diminish carbohydrate content
- When a sample preparation protocol calls for a dilution, the two parts are stated like a ratio, but what is needed is a fraction. For example, “Dilute 1:2,” means to take 1 part of one reagent and mix with 1 part of another, essentially diluting the part by half. “Dilute 1:4,” means to take 1 part and mix with 3 parts, making a total of 4 parts, diluting the part by a quarter

Preparation for PAGE

- Prepare SDS-PAGE sample buffer without reducing agent, then aliquot and store at room temperature
- Prepare fresh reducing agent, and add it to SDS-PAGE sample buffer immediately before use
- Dissolve dry protein samples directly in 1x sample buffer; prepare other protein samples such that the final sample buffer concentration is 1x
- Incubate samples in sample buffer at 95°C for 5 min (or at 70°C for 10 min) after addition of sample buffer for more complete disruption of molecular interactions
- When preparing SDS-PAGE sample buffer, use either 5% (~100 mM) 2-mercaptoethanol (βME) or 5–10 mM dithiothreitol (DTT)
- The final protein concentration in the sample solution for 1-D electrophoresis should not be <0.5 mg/ml

Protocols

Sample Preparation

Human Cells

This protocol uses sonication and radioimmunoprecipitation assay (RIPA) buffer, for cell lysis and protein extraction.

Suspension Cultured Cells

1. Pellet the cells by centrifugation at 2,000 × g for 5 min at 4°C.
2. Discard the supernatant and wash pelleted cells in cold PBS. Repeat steps 1 and 2 twice.
3. Add RIPA buffer to the pelleted cells and suspend the pellet with a pipet.

Monolayer Cultured Cells

1. Carefully remove (decant) culture medium from cells. Wash cells twice in cold PBS.
2. Add RIPA buffer to the cells and keep on ice for 5 min. Swirl the plate occasionally to spread the buffer around the plate.
3. Use a cell scraper to collect the lysate and transfer to a microcentrifuge tube.

4. Place the cell suspension on ice, incubate 5 min, and sonicate at appropriate intervals. Check lysis efficacy by light microscopy.
5. Centrifuge cell debris at ~14,000 × g for 15 min at 4°C and transfer supernatant to a new vial.
6. Perform a protein assay of the supernatant. A protein concentration of 3–5 µg/µl is best for PAGE.
7. Add 2x SDS-PAGE sample buffer to the protein solution to yield a 1x sample buffer concentration.

Reagents

- Phosphate buffered saline (PBS)
- RIPA solubilization buffer (use 1 ml RIPA buffer with 3 × 10^7 cells; store and use RIPA buffer at 4°C
- SDS-PAGE sample buffer (2x)

Equipment

- Centifuge
- Sonicator

Links

DC Protein Assay

RC DC Protein Assay

SDS-PAGE Sample Buffer

ReadyPrep 2-D Cleanup Kit

Protocols

Sample Preparation

Tips

- Use liquid nitrogen and a mortar and pestle to grind the samples while they are still frozen. Mill any larger pieces beforehand (for example, wrap the frozen tissue sample in aluminum foil and crush with a hammer)
- With plant leaves, precipitate proteins with 20% TCA in prechilled acetone (–20°C). To remove the plant phenols, rinse the pellet at least twice with cold acetone (–20°C) and air-dry samples in a vacuum

Reagents

Mammalian Tissue
- Lysis buffer
- SDS-PAGE sample buffer (2x)

Plant Leaves
- Protein precipitation solution
- Wash solution
- SDS-PAGE sample buffer (1x)

Equipment

- Mortar and pestle

Links

SDS-PAGE Sample Buffer

Mammalian Tissue

This protocol involves freezing mammalian tissue samples (for example, biopsy samples) in liquid nitrogen at –196°C.

1. Chill a mortar with liquid nitrogen, then grind small tissue pieces in the presence of liquid nitrogen to a fine powder.
2. Immediately after grinding, transfer 60 mg tissue powder to a microcentrifuge tube containing 1.0 ml lysis buffer.
3. Optional: sonicate the sample on ice 5 times, for 2 sec every time. Pause between sonication steps to avoid overheating.
4. Incubate the sample at room temperature for 30 min. Vortex from time to time.
5. Centrifuge at 35,000 x g for 30 min at room temperature.
6. Perform a protein assay to determine the protein concentration of the supernatant, which should be 5–10 µg/µl.
7. Dilute supernatant with 2x SDS-PAGE sample buffer (to a final 1x concentration), and incubate for 20 min at room temperature. Do not heat the sample.

Plant Leaves

To minimize the deleterious effects of endogenous plant compounds, use the below protocol, which involves grinding the tissue in a mortar and pestle with liquid nitrogen.

1. Cool protein precipitation and wash solutions to –20°C. Chill a mortar with liquid nitrogen.
2. Place 3–4 leaves in the mortar, add liquid nitrogen, and grind the leaves in the liquid nitrogen to a fine powder.
3. Transfer leaf powder into 20 ml of protein precipitation solution and incubate for 1 hr at –20°C. Stir occasionally.
4. Centrifuge the solution at –20°C for 15 min at 35,000 x g. Discard supernatant, add wash solution and suspend the pellet. Incubate for 15 min at –20°C, stirring occasionally. Repeat until wash solution turns light green.
5. Centrifuge the solution at –20°C for 15 min at 35,000 x g and discard supernatant. Resuspend pellet in 2 ml wash solution.
6. Transfer suspension into a shallow ceramic shell and cover with perforated parafilm. Put shell into a dessicator and apply a vacuum until the pellet (acetone powder) is dry.
7. Mix 3 mg of sample powder with 1 ml 1x SDS-PAGE sample buffer and incubate for 30 min at room temperature. Vortex from time to time.
8. Centrifuge the solution at –20°C for 15 min at >16,000 x g. Collect and heat the supernatant for 3 min at 95°C.
9. Cool solution to room temperature and perform the protein assay.

Protocols

Sample Preparation

Microbial Cultures

This protocol relies on cell lysis with ultrasonic waves in combination with a solubilization in SDS under elevated temperature. This ensures deactivation and denaturation of proteases.

1. Centrifuge cells (~5 x 10^7) for 3 min at 5,000 x g and resuspend the pellet in an equal volume of 37°C PBS and centrifuge again. Repeat two more times to remove all interfering material (extracellular proteases and growth media).
2. Add 200 µl of hot (95°C) SDS sample solubilization buffer to the pellet and vortex thoroughly.
3. Sonicate the sample solution 10 times for 1 sec each at ~60 W and ~20 kHz. Incubate at 95°C for 5 min.
4. Cool the sample to 20°C and dilute with ~250 µl 2x SDS-PAGE sample buffer. Incubate for another 20 min at room temperature.
5. Centrifuge the sample solution at 20°C for 30 min at 14,000 x g and harvest the supernatant.
6. Perform the protein assay. The protein concentration should be ~5 µg/µl.

Protein Fractions from Chromatography

When checking fraction purity or the enrichment of a particular protein after a chromatographic separation, you can observe the presence of high concentrations of salt, detergent, denaturants, and organic solvents. For example, in ion exchange chromatography, proteins are eluted by a salt gradient. But the salt concentration of the corresponding fractions can be as high as 0.5 M, a concentration that interferes with SDS-PAGE.

Remove salt and other contaminants by one of the following approaches:

- Buffer exchange — use Bio-Spin® or Micro Bio-Spin™ Columns, which are filled with size exclusion media equilibrated in Tris buffer. These columns accommodate a sample volume 50–100 µl and remove compounds <6 kD within 10 min. Mix the purified sample with 2x SDS-PAGE sample buffer
- Precipitation — use the ReadyPrep™ 2-D Cleanup Kit (based on an acetone/TCA precipitation) for simultaneous removal of interfering substances and concentration of dilute samples (<50 ng/ml)

Tips

- Reproducible lysis and protein solubilization of bacteria and yeast is challenging because the cells may release proteases and other enzymes into the growth medium. Wash the cultures thoroughly with isotonic buffers and take precautions to inactivate the proteolytic activity after cell lysis. Extensive disruption of microbial cells is required, usually with the help of a French press, bead impact instruments, or sonicator

Reagents

- SDS sample solubilization buffer
- SDS-PAGE sample buffer (2x)
- Phosphate buffered saline (PBS)

Equipment

- Centifuge
- Sonicator

Links

SDS-PAGE Sample Buffer (2x)

Bio-Spin and Micro Bio-Spin Columns

ReadyPrep 2-D Cleanup Kit

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Protocols

Sample Quantitation (*RC DC* Protein Assay)

The *RC DC* Protein Assay is based on a modification of the Lowry protocol (Lowry et al. 1951), and is both reducing agent compatible (RC) and detergent compatible (DC). Protein quantitation can be performed in complex mixtures including Laemmli buffer. It involves addition of detection reagents to a protein solution and subsequent measurement of absorbance at 750 nm with a spectrophotometer. Comparison to a standard curve provides a relative measurement of protein concentration.

Tips

- Prepare a standard curve each time the assay is performed
- For best results, prepare the standards in the same buffer as the sample

Standard Assay Protocol (5 ml)

1. Add 20 µl of *DC* Reagent S to each 1 ml of *DC* Reagent A needed. This solution is referred to as Reagent A'. Each standard or sample assayed requires 510 µl Reagent A'.
2. Prepare 3–5 dilutions of a protein standard (0.2–1.5 mg/ml protein).
3. Pipet 100 µl of protein standard or sample into clean tubes. Add 500 µl of *RC* Reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.
4. Add 500 µl of *RC* Reagent II to each tube and vortex. Centrifuge the tubes at 15,000 x g for 3–5 min.
5. Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
6. Add 510 µl of Reagent A' to each tube and vortex. Incubate tubes at room temperature for 5 min, or until the precipitate is dissolved. Vortex.
7. Add 4 ml of *DC* Reagent B to each tube and vortex immediately. Incubate at room temperature for 15 min.

Microfuge Tube Assay Protocol (1.5 ml)

1. Add 5 µl of *DC* Reagent S to each 5 µl of *DC* Reagent A needed. This solution is referred to as Reagent A'. Each standard or sample assayed requires 127 µl Reagent A'.
2. Prepare 3–5 dilutions of a protein standard (0.2–1.5 mg/ml protein).
3. Pipet 25 µl of protein standard or sample into clean microcentrifuge tubes. Add 125 µl of *RC* Reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.
4. Add 125 µl of *RC* Reagent II into each tube and vortex. Centrifuge the tubes at 15,000 x g for 3–5 min.
5. Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
6. Add 127 µl of Reagent A' to each tube and vortex. Incubate tubes at room temperature for 5 min, or until the precipitate is dissolved. Vortex.
7. Add 1 ml of *DC* Reagent B to each tube and vortex immediately. Incubate at room temperature for 15 min.

8. Read absorbance of each sample at 750 nm. The absorbances are stable for at least 1 hr.
9. Plot absorbance measurements as a function of concentration for the standards.
10. Interpolate the concentration of the protein samples from the plot and sample absorbance measurements.

Links

Criterion Cell

Mini-PROTEAN Cell

PROTEAN II xi Cell

RC DC Protein Assay

Protocols

Handcasting Polyacrylamide Gels

Single-Percentage Gels

1. Prepare the resolving and stacking gel solutions without APS or TEMED. (Tables 1 and 2; consult the instruction manual for the system you are using for more details.)

Table 1. Volume of resolving gel solution required to fill a gel cassette. Volumes listed are required to completely fill a gel cassette. Amounts may be adjusted depending on the application (with or without comb, with or without stacking gel, etc.).

Spacer (Gel Thickness)	Mini-PROTEAN®* Cell	Criterion™ Cell	PROTEAN® II xi Cell	
			16 cm	20 cm
0.5 mm	—	—	12.8 ml	16.0 ml
0.75 mm	4.2 ml	—	19.2 ml	24.0 ml
1.0 mm	5.6 ml	15.0 ml	25.6 ml	32.0 ml
1.5 mm	8.4 ml	—	38.4 ml	48.0 ml
3.0 mm	—	—	76.8 ml	96.0 ml

* 10 ml of monomer solution is sufficient for two stacking gels of any thickness.

Table 2. Recipes for stacking and resolving gels. Adjust amounts as needed for the format you are using (see Table 1).

	Stacking Gel	Resolving Gel		
	4%	7.5%	12%	X%
30% Acrylamide/bis	1.98 ml	3.75 ml	6.0 ml	0.33 x X ml
0.5M Tris-HCl, pH 6.8	3.78 ml	—	—	—
1.5M Tris-HCl, pH 8.8	—	3.75 ml	3.75 ml	3.75 ml
10% SDS	150 µl	150 µl	150 µl	150 µl
diH_2O	9 ml	7.28 ml	5.03 ml	11.03–(0.33 x X) ml
TEMED	15 µl	7.5 µl	7.5 µl	7.5 µl
10% APS	75 µl	75 µl	75 µl	75 µl
Total volume	15 ml	15 ml	15 ml	15 ml

2. Degas the solution under a vacuum for at least 15 min. While solutions are degassing, assemble the glass cassette sandwich.
3. Place a comb into the assembled gel sandwich. With a marker, place a mark on the glass plate 1 cm below the teeth of the comb. This will be the level to which the separating gel is poured. Remove the comb.

General Tips for Handcasting

- Acrylamide and bisacrylamide are neurotoxins when in solution. Avoid direct contact with the solutions and clean up spills
- For casting multiple gels, use the Mini-PROTEAN 3 Multi-Casting Chamber (catalog #1654110), PROTEAN II Multi-Gel Casting Chamber (catalog #1652025), or PROTEAN Plus Multi-Casting Chamber (catalog #1654160)
- Use only high-quality reagents, especially acrylamide monomers, to avoid polymerization problems
- Proper degassing and filtering of the casting solution is critical for both reproducibility of the polymerization (oxygen removal) and the avoidance of problems related to mass spectrometry (keratin contamination)
- A temperature of 23–25°C is best for degassing and polymerization; equilibrate the stock solutions to room temperature
- APS/TEMED-initiated reactions should proceed for at least 2 hr to ensure maximum reproducibility of pore size
- Make fresh APS solution every day for best performance
- Replace TEMED every three months because it is subject to oxidation, which causes the gradual loss of catalytic activity
- The glass plates must be clean and free of chips. Clean glass plates with ethanol and lint-free cloths before use
- The height of the stacking gel should be at least 2x the height of the sample in the well. This ensures band sharpness, even for diluted protein samples
- Store gels flat in the fridge at 4°C. Do not freeze. Wrap handcast gels tightly in plastic wrap with combs still inserted
- Run handcast gels with discontinuous buffer systems right after gel casting because the buffer discontinuity (pH and ionic strength) gradually disappears during gel storage. SDS-PAGE gels are not stable at pH 8.8 over a longer time period
- For more information about acrylamide polymerization, refer to Acrylamide Polymerization – A Practical Approach, bulletin 1156

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Protocols

Handcasting Polyacrylamide Gels

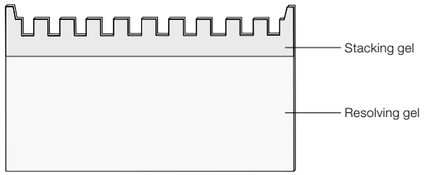
Single-Percentage Gels (contd.)

Pour the Resolving Gel

1. Add the APS and TEMED to the degassed resolving gel solution, and pour the solution to the mark, using a glass pipet and bulb.
2. Using a Pasteur pipet and bulb, immediately overlay the monomer solution with water-saturated *n*-butanol.
3. Allow the gel to polymerize 45–60 min. The gel is polymerized once you see a line form between the stacking and the resolving gel. Pour off the overlay solution and rinse the top of the gel with diH_2O.

Alternative Casting Procedure

It is possible to cast separation and stacking gels one after another, with no intermediate step requiring overlay solution (water-saturated *n*-butanol). Recalculate your gel casting recipes so that the separation gel solution contains 25% (w/v) glycerol. Due to the significant difference in density, the two solutions won't mix when the stacking gel solution is carefully poured on top of the resolving gel solution.



Pour the Stacking Gel

1. Dry the area above the separating gel with filter paper before pouring the stacking gel.
2. Place the comb in the cassette and tilt it so that the teeth are at a slight (~10°) angle. This prevents air from becoming trapped under the comb while the acrylamide solution is being poured.
3. Add the APS and TEMED to the degassed resolving gel solution, and pour the solution down the spacer nearest the upturned side of the comb. Pour until all the teeth are covered by the solution.
4. Realign the comb in the sandwich and add monomer to fill the cassette completely. An overlay solution is not necessary for polymerization when a comb is in place.
5. Allow the gel to polymerize 30–45 min.
6. Remove the comb by pulling it straight up slowly and gently. Rinse the wells completely with diH_2O.

Tips

- When pouring the resolving gel solution, pour the solution down the middle of the outside plate of the gel sandwich or down the side of one of the spacers. Pour smoothly to prevent it from mixing with air
- For the overlay solution, water, *n*-butanol, or t-amyl alcohol can also be used. With *n*-butanol or t-amyl alcohol, the overlay solution can be applied rapidly because very little mixing will occur. If using water to overlay, use a needle and syringe and a steady, even rate of delivery to prevent mixing
- Do not allow alcohols to remain on the gels for more than 1 hr or dehydration of the top of the gel will occur. It is sometimes convenient to cast the separating portion of the discontinuous gel the afternoon before casting the stacking gel and running the gel. If the stacking gel is to be cast the following day, place approximately 5 ml of 1:4 diluted running gel buffer on top of each separating gel after rinsing with deionized water to prevent dehydration of the separating gel

Links

Polyacrylamide Gel Reagents

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Protocols

Handcasting Polyacrylamide Gels

Gradient Gels

This protocol is for preparing 12 mini-format linear gradient gels. It requires the Model 485 Gradient Former and Mini-PROTEAN 3 Multi-Casting Chamber. For other protocols, refer to the instruction manual for the gradient former you are using.

1. Determine the volume of acrylamide to prepare (≥40 ml is required for the Model 485 Gradient Former). Prepare the required volume (+5 ml) of acrylamide. Table 3 provides estimated volumes for the casting of 12 mini-format gels. Assemble the stack of Mini-PROTEAN 3 Cassettes as described in the Mini-PROTEAN 3 Multi-Casting Chamber instruction manual. Then, flow water through the stopcock and measure the volume required to fill the cassettes. Disassemble the chamber and dry all components.

Table 3. Volume of acrylamide required for 12 mini-format gels. Prepare the amount listed below plus an additional 5 ml.

Spacer Plates	Volume Required for 12 Gels	Volume to Prepare
0.75 mm	80 ml	85–90 ml
1.0 mm	100 ml	105–110 ml
1.5 mm	140 ml	145–150 ml

Table 4. Preparation of light and heavy acrylamide solutions.

Light Solution (4%)	
30% Acrylamide stock (30%)(X ml) = (4%)(55 ml)	X = 7.3 ml
1.5 M Tris-HCl stock buffer, pH 8.8 (1.5 M)(X ml) = (0.375 M)(55 ml)	X = 13.8 ml
Water (55 ml) – (7.3 ml + 13.8 ml) = X	X = 34 ml
10% APS (500 µl)/(100 ml) = (X µl)/(55 ml)	X = 275 µl
TEMED 10% APS volume; (275 µl)/10 = X	X = 27.5 µl
Heavy Solution (20%)	
30% Acrylamide stock (30%)(X ml) = (20%)(55 ml)	X = 36.7 ml
1.5 M Tris-HCl stock buffer, pH 8.8 (1.5 M)(X ml) = (0.375 M)(55 ml)	X = 13.8 ml
Water (55 ml) – (36.7 ml + 13.8 ml) = X	X = 4.5 ml
APS (500 µl)/(100 ml) = (X µl)/(55 ml)	X = 275 µl
TEMED 10% APS volume; (275 µl)/10 = X	X = 27.5 µl

2. Determine the chamber volumes. To create a linear gradient, the volume in each chamber is half the total gel volume required (or 20 ml, whichever is greater). As an example, casting twelve 1.0 mm gels requires 100 ml, so prepare 105 ml (step 1). Divide that volume by two to determine the volume required for each chamber of the gradient former (52.5 ml each for the light and heavy chambers).
3. Determine the heavy and light acrylamide formulations using the chart on the right. (see Table 4) Reassemble the multi-casting chamber.
4. Place the gradient former on a magnetic stir plate and add a magnetic stir bar to the mixing chamber labeled "light." Attach the luer fitting to the stopcock valve on the inlet port. Run a piece of Tygon tubing (1/8" ID Tygon tubing works well) from the gradient former to the luer fitting on the multi-casting chamber.
5. Combine all reagents except the initiators, and degas the solution for 15 min.
6. Just prior to pouring, add TEMED and APS to both solutions and mix gently. Pour the appropriate monomer solutions into the gradient chambers. (Consult the gradient former instruction manual for complete instructions.) Pour the light solution into the mixing chamber labeled "light," and the heavy solution in the reservoir chamber labeled "heavy."
7. Turn on the stirring bar in the mixing chamber, open the tubing clamp of the gradient maker and the stopcock valve of the casting chamber, and pour the gels.

Tips

- If gravity flow isn't fast enough, use a peristaltic pump to pump the entire set of gradients within 10 min. If it is not possible to complete the operation in 10 min from the time initiators are added, then it might be necessary to reduce the amount of initiators (use half the amount of TEMED) to slow polymerization. The gradient should be poured as quickly as possible, without mixing the gradient solution in the casting chamber

Links

Mini-PROTEAN 3 Multi-Casting Chamber

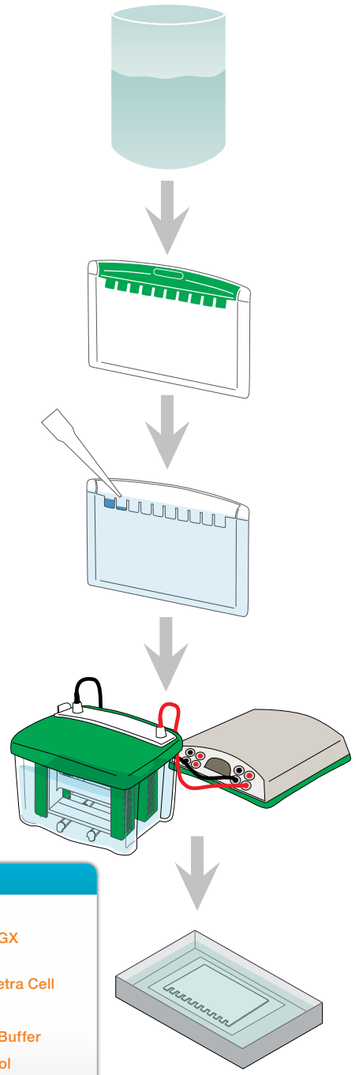
Model 485 Gradient Former

Protocols

Performing Electrophoresis

General Protocol: SDS-PAGE

The following is a generalized protocol for running a Mini-PROTEAN® TGX™ Gel in the Mini-PROTEAN Tetra Cell. For detailed instructions, refer to the Mini-PROTEAN Precast Gels instruction manual and application guide (bulletin #1658100).



1

Prepare buffers:

a. Running buffer (1x): Dilute 100 ml of 10x stock with 900 ml diH_2O.

b. Sample buffer: Use Laemmli sample buffer.

2

Prepare gels and assemble the electrophoresis cell:

a. Remove the comb and tape from the gels and assemble the electrophoresis cell.

b. Fill the inner and outer buffer chambers with running buffer. Fill the upper (inner) buffer chamber of each core with 200 ml of 1x running buffer. Fill the lower (outer) buffer chamber to the indicator mark for 2 gels (550 ml) or 4 gels (800 ml) with 1x of running buffer. At runs >200 V, fill the outer buffer chamber to the 4-gel (800 ml) mark.

3

Prepare samples as indicated in the table below.

Component	Reducing	Nonreducing
Sample	5 µl	5 µl
Laemmli sample buffer	4.75 µl	5 µl
β-mercaptoethanol	0.25 µl	—
Total volume	10 µl	10 µl

4

Heat samples at 90–100°C for 5 min (or at 70°C for 10 min). Load the appropriate volume of your protein sample on the gel.

5

Connect the electrophoresis cell to the power supply and perform electrophoresis according to the following conditions:

Run conditions: 200 V
Run time: 31–39 min
Expected current (per gel): Initial 35–50 mA
Final 20–31 mA

These conditions are for Tris-HCl SDS-PAGE gels. If using Bio-Rad's Mini-PROTEAN TGX Gels, the gels can be run at 300 V to decrease the run time.

6

After electrophoresis is complete, turn the power supply off and disconnect the electrical leads. Pop open the gel cassettes and remove the gel by floating it off the plate into water.

7

Stain and image the gel, using one of the protocols on the following pages as examples.

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Links

Mini-PROTEAN TGX Precast Gels
Mini-PROTEAN Tetra Cell
Running Buffer
Laemmli Sample Buffer
β-Mercaptoethanol
PowerPac Basic Power Supply

Protocols

Performing Electrophoresis

Tips for Electrophoresis

- When preparing running buffers, make the solution as specified in the protocol and do not titrate to a pH. The ion balance is set by the concentration of reagents; adjusting the pH alters this balance and leads to undesirable results
- Do not reuse running buffers
- Use 5–10 V per cm of gel for about 10 min during sample entry (or until the sample has concentrated at the starting point of the separation gel). Then continue with the voltage setting recommended in the instruction manual for the electrophoresis system you are using
- Use the voltage setting recommended in the instruction manual for the electrophoresis system you are using; excessive voltage leads to decreased band resolution, band smiling, and lane distortions
- When running multiple cells, use the same voltage for multiple cells as you would for one cell. Be aware that the current drawn by the power supply will double with two – compared to one – cells. Use a power supply that can accommodate this additive current and set the current limit high enough to permit this additive function
- To maximize reproducibility, maintain the temperature of the electrophoresis buffer at 15°C with the help of a recirculating cooler

Tips for Sample Loading

- The total protein amount loaded per lane depends on the sample complexity and sensitivity of the staining technique. Using 15–20 µg protein per lane for mini- or midi-format gels is a good starting point for complex protein samples when staining with Bio-Safe™ Coomassie Stain. Determine the optimum protein load by running a dilution series of the sample
- For best resolution, load a concentrated sample rather than a diluted amount
- Centrifuge the sample solution for 10–15 min at >12,000 x g at 20°C before loading to remove insoluble material that may clog the pores of the acrylamide gel
- To avoid edge effects, add 1x sample buffer to unused wells and never overfill wells
- Load samples either before or after placing the electrophoresis modules into the tank. Both methods produce acceptable results. In both cases, fill both the assembly (inner chamber) and the tank (outer chamber) with buffer
- Add running buffer to the cathode buffer reservoir first and then apply the sample on the stacking gel under the electrode buffer. Sample buffer must contain glycerol to stabilize the sample application zone in the sample well of the gel
- Use pipet tips designed for protein sample loading for best results. For example, Bio-Rad's Prot/Elec™ Tips fit easily between vertical slab gel plates of 0.75 mm while maintaining a large bore for fast flow of sample
- Load samples slowly to allow them to settle evenly on the bottom of the well. Be careful not to puncture the bottom of the well with the syringe needle or pipet tip
- If using Bio-Rad's patented sample loading guide, place it between the two gels in the electrode assembly. Sample loading guides are available for 9, 10, 12, and 15-well formats. Use the sample loading guide to locate the sample wells. Insert the Hamilton syringe or pipet tip into the slots of the guide and fill the corresponding wells

Links

Prot/Elec Tips

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TIPS

- Always wear gloves during the staining process. Try to avoid touching the gels with your fingers. Wet gloves with water or buffer before handling the gel to keep the gel from sticking and tearing
- Use clean and dust-free containers for gel staining. If possible, place a lid on the container to avoid contamination of the staining solution
- Gently agitate the container on a horizontal shaker, making sure the gel is completely covered with stain solution all the time
- Use pure chemicals and highly purified diH_2O (conductivity <2 µS)
- Fluorescent dyes like Flamingo and Oriole Fluorescent Gel Stains have a higher dynamic range than Coomassie or silver staining techniques, making them suitable for quantitative protein analysis
- Gels stained with fluorescent dyes can be counterstained with colloidal Coomassie for further reference. In fact, doing so enhances sensitivity of the colloidal Coomassie Stain
- For long term-storage, shrink-wrap the stained gels in a 10% glycerol solution (storage at 4°C)

Links

Mini-PROTEAN Gels
Coomassie Stains
Bio-Safe Coomassie Stain
Fluorescent Protein Stains
Flamingo Fluorescent Gel Stain
Oriole Fluorescent Gel Stain
Silver Stains

Protocols

Total Protein Staining

General protocols are described below for Mini-PROTEAN Gels. For more details, refer to the instruction manual for the stain you are using.

Bio-Safe™ Coomassie Stain

1. Wash gels three times for 5 min each in 200 ml diH_2O per gel.
2. Remove all water from staining container and add 50 ml of Bio-Safe Coomassie Stain (or enough to completely cover gel). Agitate for 1 hr.
3. Rinse in 200 ml diH_2O for ≥30 min. Stained gels can be stored in water.

Oriole™ Fluorescent Gel Stain

1. If using the 5 L configuration, prepare Oriole Stain solution by adding 400 ml of methanol to the 1 L bottle of diluents. Then add 10 ml of Oriole Fluorescent Gel Stain concentrate and mix well by shaking.
2. Place gel in a staining tray with 50 ml of Oriole Fluorescent Gel Stain. Cover the tray, place on a rocker, and agitate gently for ~1.5 hr.
3. Transfer the gel to diH_2O prior to imaging. Destaining is not necessary.

Flamingo™ Fluorescent Gel Stain

1. Place gel in a staining tray with 100 ml of fixing solution (40% ethanol, 10% acetic acid). Cover the tray, place on a rocker, and agitate gently for at least 2 hr.
2. Pour off the fix solution and add 50 ml of 1x stain solution (dilute 1 part Flamingo Fluorescent Gel Stain with 9 parts diH_2O). Cover the tray, place on a rocker or shaker and agitate gently. Stain for at least 3 hr.
3. (Optional) Carefully pour off the stain solution and replace with an equal volume of 0.1% (w/v) Tween 20. Cover the tray, place on a rocker or shaker and agitate gently for 10 min.
4. Rinse gel with diH_2O prior to imaging.

Silver Staining (Bio-Rad Silver Stain)

Step	Reagent	Volume	Duration		
			<0.5 mm Gel	0.5–1.0 mm Gel	>1.0 mm Gel
1	Fixative 40% methanol/10% acetic acid	400 ml	30 min	30 min	60 min
2	Fixative 10% ethanol/5% acetic acid	400 ml	15 min	15 min	130 min
3		400 ml	15 min	15 min	130 min
4	Oxidizer	200 ml	3 min	5 min	10 min
5	diH_2O	400 ml	2 min	5 min	10 min
6		400 ml	2 min	5 min	10 min
7	(Repeat washes 5–7 times until all the yellow color is gone from the gel)	400 ml	2 min	5 min	10 min
8	Silver reagent	200 ml	15 min	20 min	30 min
9	diH_2O	400 ml	—	1 min	2 min
10	Developer	200 ml	~30 sec. Develop until solution turns yellow or until brown precipitate appears.		
11		200 ml	~5 min	~5 min	~5 min
12		200 ml	—	~5 min	~5 min
13	Stop 5% acetic acid	400 ml	~5 min	~5 min	~5 min

Links

Image Lab Software
Precision Plus Protein Unstained Standards
Criterion Tris-HCl Precast Gels
Bio-Safe Coomassie Stain

Protocols

Molecular Weight Estimation

Run the standards and samples on an SDS-PAGE gel. Process the gel with the desired stain and then destain to visualize the protein bands. Determine the R_f graphically or using Image Lab™ Software (or equivalent).

Figures 1 and 2 illustrate the procedure.
To determine MW graphically:

1. Using a ruler, measure the migration distance from the top of the resolving gel to each standard band and to the dye front.
2. For each band in the standards, calculate the R_f value using the following equation:

   R_f = migration distance of the protein/ migration distance of the dye front
3. Repeat this step for the unknown bands in the samples.
4. Using a graphing program, plot the log (MW) as a function of R_f.
5. Generate the equation y = mx + b, and solve for y to determine the MW of the unknown protein.

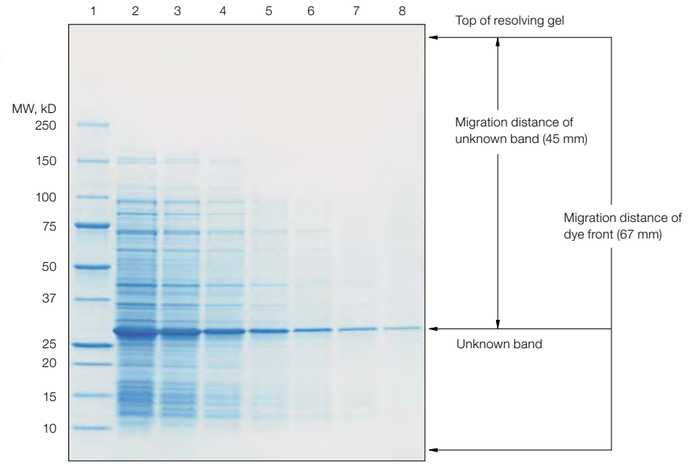

Fig. 1. Example showing MW determination of an unknown protein. Lane 1, 10 µl of Precision Plus Protein™ unstained standards; lanes 2–8, a dilution series of an *E. coli* lysate containing a hypothetical unknown protein (GFP). Proteins were separated by SDS-PAGE in a Criterion™ 4–20% Tris-HCl Gel and stained with Bio-Safe Coomassie stain. Gel shown is the actual size.

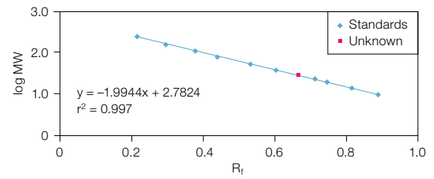

Fig. 2. Determining the MW of an unknown protein by SDS-PAGE. A standard curve of the log (MW) versus R_f was generated using the Precision Plus Protein standards from Figure 1. The strong linear relationship ($r^2 > 0.99$) between the proteins' MW and migration distances demonstrates exceptional reliability in predicting MW.

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Buffer Formulations

Sample Preparation Buffers

1 M Tris-HCl, pH 7.6 (100 ml)

Tris base	12.11 g
Deionized H_2O (diH_2O)	80 ml
Adjust pH to 7.6 with HCl.	
diH_2O	to 100 ml

0.5 M Tris-HCl, pH 6.8 (100 ml)
(catalog #1610799)

Tris base	6.06 g
diH_2O	~60 ml
Adjust to pH 6.8 with HCl.	
diH_2O	to 100 ml
Store at 4°C.	

10% SDS (10 ml)
(catalog #1610416)

SDS	1.00 g
diH_2O	to 10 ml

1.0% Bromophenol Blue (10 ml)
(catalog #1610404)

Bromophenol blue	100 mg
diH_2O	to 10 ml

RIPA Solubilization Buffer (100 ml)
25 mM Tris-HCl pH 7.6, 150 mM NaCl, 5 mM EDTA, 1% NP-40 or 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS

NaCl	0.88 g
EDTA	0.15 g
NP-40 or Triton X-100	1 g
Sodium deoxycholate	1 g
SDS	0.10 g
diH_2O	80 ml
1 M Tris-HCl, pH 7.6	2.5 ml
diH_2O	to 100 ml

Phosphate Buffered Saline (PBS, 1 L)
0.9% (w/v) sodium chloride in 10 mM phosphate buffer, pH 7.4

NaCl	8.00 g
KCl	0.20 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g
diH_2O	800 ml
Adjust pH to 7.4 with HCl or NaOH.	
diH_2O	to 1 L

SDS-PAGE Sample Buffer (2x, 8 ml)
(catalog #1610737, 30 ml)
62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue

0.5 mM Tris-HCl, pH 6.8	1.0 ml
25% Glycerol	2.0 ml
1.0% Bromophenol blue	0.08 ml
10% SDS	1.6 ml
diH_2O	2.92 ml

Store as 1–2 ml aliquots at –70°C and add β-mercaptoethanol (0.4 ml) or 3% DTT immediately before use.

Protein Precipitation Solution (100 ml)
20% Trichloroacetic acid (TCA), 0.2% DTT in ice-cold acetone (–20°C)

TCA	20.00 g
DTT	0.20 g
Acetone	80 ml
Dissolve	
Acetone	to 100 ml
Store at –20°C.	

Wash Solution (100 ml)
0.2% DTT in ice-cold acetone (–20°C)

DTT	0.20 g
Acetone	80 ml
Dissolve	
Acetone	to 100 ml
Store at –20°C.	

Lysis Buffer (50 ml)
2 M thiourea, 7 M urea, 4% (w/v) CHAPS, 1% (w/v) DTT, 2% (v/v) carrier ampholytes (pH 3–10)

Urea	21.00 g
Thiourea	7.60 g
diH_2O	to 45 ml
CHAPS	2.00 g
Bio-Lyte® Ampholytes	1.0 ml
DTT	0.50 g
diH_2O	to 50 ml

SDS Sample Solubilization Buffer (50 ml)
1% (w/v) SDS, 100 mM Tris-HCl (pH 9.5)

SDS	0.50 g
Tris base	0.60 g
diH_2O	40 ml
Titrate to pH 9.5 with diluted HCl.	
diH_2O	to 50 ml

Buffer Formulations

Gel Casting Reagents

Acrylamide/Bis (30% T, 2.67% C)

Acrylamide (29.2 g/100 ml)	87.60 g
N'N'-bis-methylene-acrylamide	2.40 g
diH_2O	to 300 ml
Filter and store at 4°C in the dark (30 days).	

Premade alternatives:
Catalog #161-0125, 37.5:1 acrylamide/bis powder
Catalog #161-0158, 30% acrylamide/bis solution

1.5 M Tris-HCl, pH 8.8 (150 ml)
(catalog #1610798, 1 L)

Tris base (18.15 g/100 ml)	27.23 g
diH_2O	80 ml
Adjust to pH 8.8 with 6 N HCl.	
diH_2O	to 150 ml
Store at 4°C.	

0.5 M Tris-HCl, pH 6.8
(catalog #1610799, 1 L)

Tris base	6.00 g
diH_2O	60 ml
Adjust to pH 6.87 with 6 N HCl.	
diH_2O	to 100 ml
Store at 4°C.	

10% (w/v) SDS (100 ml)
(catalog #1610416)

SDS	10.00 g
diH_2O	90 ml
Dissolve with gentle stirring	
diH_2O	to 100 ml

10% (w/v) APS (fresh daily)
(catalog #1610700)

Ammonium persulfate	0.10 g
diH_2O	1 ml

Water-Saturated *n*-Butanol

n-Butanol	50 ml
diH_2O	5 ml

Combine in a bottle and shake. Use the top phase only. Store at room temperature.

Sample Buffers

2x SDS-PAGE (Laemmli, 30 ml)
(catalog #1610737)
62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol (added fresh)

0.5 M Tris-HCl, pH 6.8	3.75 ml
50% Glycerol	15.0 ml
1.0% Bromophenol blue	0.3 ml
10% SDS	6.0 ml
diH_2O	to 30 ml

Add β-mercaptoethanol (50 µl to 950 µl sample buffer) before use.

2x Native PAGE (30 ml)
(catalog #1610738)
62.5 mM Tris-HCl, pH 6.8, 40% glycerol, 0.01% bromophenol blue

0.5 M Tris-HCl, pH 6.8	3.75 ml
50% Glycerol	24.0 ml
1.0% Bromophenol blue	0.3 ml
diH_2O	to 30 ml

2x Tricine (30 ml)
(catalog #1610739)
200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Brilliant Blue G-250, 2% β-mercaptoethanol (added fresh)

1.0 M Tris-HCl, pH 6.8	6.0 ml
100% Glycerol	12.0 ml
10% SDS	6.0 ml
Coomassie blue G-250	12.0 mg
diH_2O	to 30 ml

Add β-mercaptoethanol (20 to 980 µl sample buffer) before use.

Buffer Formulations

Running Buffers

10x SDS-PAGE (1 L)
(catalog #1610732)
250 mM Tris, 1.92 M glycine, 1% SDS, pH 8.3

Tris base	30.30 g
Glycine	144.10 g
SDS	10.00 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3).

10x Native PAGE (1 L)
(catalog #1610734)
250 mM Tris, 1.92 M glycine, pH 8.3

Tris base	30.30 g
Glycine	144.10 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3).

10x Tris-Tricine (1 L)
(catalog #1610744)
1 M Tris, 1 M Tricine, 1% SDS, pH 8.3

Tris base	121.10 g
Tricine	179.20 g
SDS	10.00 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3).

Buffer Components

0.5 M Tris-HCl, pH 6.8 (1 L)
(catalog #1610799)

Tris base	60.60 g
diH_2O	~900 ml

Adjust to pH 6.8 with HCl.

diH_2O	to 1 L

Store at 4°C.

10% SDS (250 ml)
(catalog #1610416)

SDS	25.00 g
diH_2O	to 250 ml

1.0% Bromophenol Blue (10 ml)
(catalog #1610404)

Bromophenol blue	100 mg
diH_2O	to 10 ml

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Part III
Troubleshooting

Electrophoresis is a straightforward technique. However, problems may occasionally arise during the various steps in the electrophoresis workflow. This section highlights potential problems and their causes, and provides potential solutions.

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Sample Preparation

Problem	Cause	Solution
Laemmli sample buffer turns yellow	Sample buffer is too acidic	Add Tris base until buffer turns blue again
Sample very viscous	High DNA or carbohydrate content	• Fragment DNA with ultrasonic waves during cell lysis and protein solubilization • Add endonucleases (for example benzonase) • Precipitate protein with TCA/acetone (ReadyPrep™ 2-D Cleanup Kit) to diminish carbohydrate content

Gel Casting and Sample Loading

Problem	Cause	Solution
Leaking during handcasting	Chipped glass plates	Ensure plates are free of flaws
	Spacer plate and short plate not level	Ensure plates are aligned correctly
	Casting stand gasket dirty, flawed, or worn out	• Wash gasket if it is dirty • Replace flawed or worn out casting stand gaskets
Poor well formation	Incorrect catalyst used	• Prepare fresh catalyst solution • Increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED
	Monomer solution not degassed (oxygen inhibits polymerization)	Degas monomer solution immediately prior to casting stacking gel
Webbing; excess acrylamide behind the comb	Incorrect catalyst concentration	• Prepare fresh catalyst solution • Increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED
Pressure cams on casting frame are difficult to close or make noise when closed	Powder residue has built up at pivot point of pressure cams	Rinse or wipe off powder residue before each use
Gel does not polymerize	Too little or too much APS or TEMED	Use 0.05% APS and 0.05% TEMED
	Failure to degas	Degas monomer solutions 10–15 min
	Temperature too low	Cast at room temperature, warming glass plates if necessary
	Poor quality acrylamide or bis	Use electrophoresis-grade reagents
	Old APS	Prepare fresh APS

Gel Casting and Sample Loading (contd.)

Problem	Cause	Solution
Swirls in gel	Excess catalysts; polymerization time <10 min	Reduce APS and TEMED by 25% each
	Gel inhibition; polymerization time >2 hr	Increase APS and TEMED by 50%; degas
Gel feels soft	Low %T	Use different %T
	Poor quality acrylamide or bis	Use electrophoresis-grade reagents
	Too little cross-linker	Use correct %C
Gel turns white	Bis concentration too high	Check solutions or weights
Gel brittle	Cross-linker too high	Use correct %C
Sample floats out of well	Sample not dense enough	Include 10% glycerol in sample to make it denser than surrounding buffer
	Pipetting, loading error	Pipet sample into well slowly. Do not squirt sample quickly into well, as it may bounce off bottom or sides and flow into next well. Do not remove pipet tip from well before last of sample has left tip

Electrophoresis

Problem	Cause	Solution
Current zero or less than expected, and samples do not migrate into gel	Tape at the bottom of precast gel cassette not removed	Remove tape
	Insufficient buffer in inner buffer chamber	Fill buffer chamber with running buffer
	Insufficient buffer in outer buffer chamber	Fill inner and outer buffer chambers to ensure wells are completely covered
	Electrical disconnection	Check electrodes and connections
Gels run faster than expected	Running buffer too concentrated and gel temperature too high; incorrect running buffer concentration or type used	Check buffer composition and type
	Running or reservoir buffer too dilute	Check buffer protocol and concentrate if necessary
	Voltage too high	Decrease voltage by 25–50%
Gels run slower than expected	Incorrect running buffer composition or type	Check buffer composition and type
	Excessive salt in sample	Desalt sample
Buffer leaking from inner chamber	Incomplete gasket seal	Wet gasket with running buffer before use
	Improper assembly of gel into the electrode/companion assembly	• Ensure that top edge of short plate fits under notch at top of gasket • Ensure that top of short plate touches the green gasket

TIPS

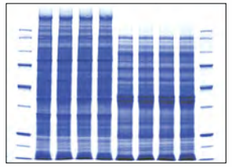

Fig. 1. Schematic of protein migration during SDS-PAGE.

Figure 1 provides an example of an optimal gel image, where the bands are nicely resolved, each lane is very straight, and protein bands are present across the length of the gel (there is excellent separation across the entire molecular weight range).

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Electrophoresis (contd.)

Problem	Cause	Solution
Leaking from upper buffer chamber (Mini-PROTEAN® Tetra Cell)	Upper buffer chamber overfilled	Keep buffer level below top of spacer plate
	Improper assembly	• Ensure that U-shaped electrode core gasket is clean, free of cuts, and lubricated with buffer • Ensure that short plate is under notch on gasket

Total Protein Staining

Problem	Cause	Solution
Bands not visible	No protein in gel	Stain with another method to confirm there is protein
	Imaging system malfunctioning	Check instrument manual for troubleshooting, or contact imaging instrument manufacturer
	Incorrect imaging parameters were used	Check instrument manual
Poor staining sensitivity	Dirty staining trays	Clean staining trays and other equipment with laboratory glassware cleaner
	Insufficient stain volume	Follow recommendations for stain volume (appropriate to gel size)
	Insufficient staining time	Increase staining time
	Reuse of staining solution	Repeat staining protocol with fresh staining solution
High or uneven background staining	Dirty equipment or staining trays	Clean staining trays and other equipment with laboratory glassware cleaner
	Too much time in staining solution	• Restrict duration of incubation in staining solutions as recommended in protocol • Wash gel in water or respective destaining solution for ≥30 min
	Reagent impurities	Use high-purity water and reagents for staining
Speckles or blotches in gel image	Particulate material from reagents, staining tray, dust, or gloves	• Clean staining trays thoroughly • Limit time that gels and staining solution are exposed to open air • Use dust-free gloves and handle gels only by edges
Uneven staining	Insufficient shaking during staining	Agitate gel during staining
Gel shrinkage	Gel dehydrated	Transfer gel to water for rehydration

Evaluation of Separation

Problem	Cause	Solution
Diffuse or broad bands	Poor quality acrylamide or bis-acrylamide, incomplete polymerization	• Use electrophoresis-grade reagents • Check polymerization conditions
	Old SDS or sample buffer	Prepare fresh solutions
	Gel temperature too high	Use external cooling during run or run more slowly
Bands “smile” across gel, band pattern curves upward at both sides of gel	Excess heating of gel; center of gel runs hotter than either end	• Check buffer composition; buffer not mixed well, or buffer in upper chamber too concentrated • Prepare new buffer, ensuring thorough mixing, especially when diluting 5x or 10x stock
	Power conditions excessive	Do not exceed recommended running conditions. Decrease power power setting from 200 V to 150 V or fill lower chamber to within 1 cm of top of short plate
	Insufficient buffer	Fill inner and outer buffer chambers to ensure that wells are completely covered
Smiling or frowning bands within gel lane	Overloaded proteins	Load less protein
	Sample preparation/buffer issues	Minimize salts, detergents, and solvents in sample preparation and sample buffers
	Incorrect running conditions	Use correct voltage
Skewed or distorted bands, lateral band spreading	Excess salt in samples	Remove salts from sample by dialysis or desalting column prior to sample preparation
	Ionic strength of sample lower than that of gel	Use same buffer in samples as in gel
	Insufficient sample buffer or wrong formulation	Check buffer composition and dilution instructions
	Diffusion prior to turning on current	Minimize time between sample application and power startup
	Diffusion during migration through stacking gel	• Increase %T of stacking gel to 4.5% or 5% T • Increase current by 25% during stacking
	Uneven gel interface	• Decrease polymerization rate • Overlay gels carefully • Rinse wells after removing comb to remove residual acrylamide

Evaluation of Separation (contd.)

Problem	Cause	Solution
Vertical streaking	Overloaded samples	• Dilute sample • Selectively remove predominant protein in sample (fractionate) • Reduce voltage by 25% to minimize streaking
	Sample precipitation	• Centrifuge samples to remove particulates prior to sample loading • Dilute sample in sample buffer
Fuzzy or spurious artifactual bands	Concentration of reducing agent too low	Use 5% BME or 1% DTT
Protein bands do not migrate down as expected	Bands of interest may be neutral or positively charged in buffer used; pI of bands must be ~2 pH units more negative than pH of running buffer	Use SDS-PAGE or a different buffer system in native PAGE or IEF

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Glossary

%C Cross-linker concentration; weight percentage of cross-linker in a polyacrylamide gel

%T Monomer concentration (acrylamide + cross-linker) in a gel (in g/100 ml). Gels can be made with a single, continuous %T through the gel (single-percentage gels), or with a gradient of %T through the gel (gradient gels)

2-D electrophoresis Two-dimensional electrophoresis. Proteins are separated first according to isoelectric point (pI) by isoelectric focusing (IEF) and then according to size by SDS-PAGE, yielding a two-dimensional protein map of spots

2-Mercaptoethanol Reducing agent necessary for cleavage of intra- and inter-molecular disulfide bonds to achieve complete protein unfolding and to maintain proteins in a fully reduced state. Also known as β-mercaptoethanol or BME

Acrylamide Monomer used with a cross-linker to form the matrix used for separating proteins or small DNA molecules

Ammonium persulfate (APS) Initiator used with TEMED (catalyst) to initiate the polymerization of acrylamide and bisacrylamide in making a polyacrylamide gel; $(NH_4)_2S_2O_8$

Ampholyte Amphoteric molecule (containing both acidic and basic groups) that exists mostly as a zwitterion in a certain pH range. Ampholytes are used to establish a stable pH gradient for use in isoelectric focusing

Anode Positively charged electrode. Negatively charged molecules (anions) move toward the anode, which is usually colored red

Anionic dye Negatively charged compound used as a stain; used in blotting to stain proteins immobilized on membranes such as nitrocellulose or PVDF

Antibody Immunoglobulin; protein produced in response to an antigen, which specifically binds the portion of the antigen that initiated its production

Antigen Foreign molecule that specifically binds with an antibody

Assay Analysis of the quantity or characteristics of a substance

Background Nonspecific signal or noise that can interfere with the interpretation of valid signals

Bio-Spin® Columns Family of Bio-Rad sample preparation products that includes the Bio-Spin 6 and Micro Bio-Spin™ 6 Columns used for buffer exchange and desalting applications

Bis or bis-acrylamide A common cross-linker used with acrylamide to form a support matrix; N,N'-methylene-bis-acrylamide

Blocking reagent Protein used to saturate unoccupied binding sites on a blot to prevent nonspecific binding of antibody or protein probes to the membrane

Blot Immobilization of proteins or other molecules onto a membrane, or a membrane that has the molecules adsorbed onto its surface

Blue native PAGE Discontinuous electrophoretic system that allows high-resolution separation of membrane protein complexes in native, enzymatically active states. Membrane protein complexes are solubilized by neutral, nondenaturing detergents like n-dodecyl-β-D-maltoside. After addition of Coomassie (Brilliant) Blue G-250, which binds to the surface of the proteins, separation of the negatively charged complexes according to mass is possible

Bromophenol blue Common tracking dye used to monitor the progress of electrophoresis

Carrier ampholytes Heterogeneous mixture of small (300–1,000 Da) polyamino-polycarboxylate buffering compounds that have closely spaced pI values and high conductivity. Within an electric field, they align according to pI to establish the pH

Cathode Negatively charged electrode. Positively charged molecules (cations) move toward the cathode, which is usually colored black

CHAPS Zwitterionic detergent (having both positively and negatively charged groups with a net charge of zero) that is widely used for protein solubilization for IEF and 2-D electrophoresis; 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate

Comb Object used to cast wells in an agarose or acrylamide gel. In PAGE applications, square-bottom combs are inserted into the gel sandwich before polymerization to form square-bottomed wells

Continuous buffer system Gel-based electrophoresis system that uses the same buffer (at constant pH) in the gel, sample, and electrode reservoirs. Typically a single-percentage gel is used, and the sample is loaded directly into the part of the gel in which separation occurs. Continuous systems are not common in protein separations; they are used mostly for nucleic acid analysis

Coomassie (Brilliant) Blue Anionic dye used in the total protein staining of gels and blots that comes in two forms: Coomassie (Brilliant) Blue G-250 differs from Coomassie (Brilliant) Blue R-250 by the addition of two methyl groups

Criterion™ Cells, Blotters, and Gels Family of Bio-Rad products used for midi-format vertical electrophoresis; includes the Criterion and Criterion™ Dodeca™ Cells, Criterion Blotter, and Criterion Precast Gels

Cross-linker Molecule (for example, bis-acrylamide) used to link polymerizing monomer molecules together to form the gel, a netlike structure. Holes in the nets are called pores, and pore size is determined in part by the cross-linker concentration. Pores may or may not sieve the macromolecules

***DC*™ Assay Kit** Bio-Rad's detergent-compatible protein assay kit

Discontinuous buffer system Gel-based electrophoresis system that uses different buffers and sometimes different buffer compositions to focus and separate components of a sample. Discontinuous systems typically focus the proteins into tighter bands than continuous gel systems, allowing larger protein loads

Dithiothreitol (DTT) Reducing agent necessary for cleavage of intra- and inter-molecular disulfide bonds to achieve complete protein unfolding and to maintain all proteins in a fully reduced state

Electroelution Technique that applies the principles of electrophoresis to enable recovery (elution) of molecules such as proteins from gels and gel slices

Electrophoresis Movement of charged molecules in a uniform electric field

Glycerol Small nonionic molecule used in vertical gel electrophoresis to increase the density of the sample buffer so that it sinks to the bottom of the sample well; also used to help keep proteins soluble, especially in isoelectric focusing

Glycine Amino acid used as the trailing ion in discontinuous electrophoresis

Gradient gel Gel with gradually changing monomer concentration (%T) in the direction of migration. In SDS-PAGE, gradients are used to separate wider molecular weight ranges than can be separated with single-percentage gels

Immobilized pH gradient (IPG) Strips in which buffering groups are covalently bound to an acrylamide gel matrix, resulting in stable pH gradients except the most alkaline (>12) pH values. This eliminates problems of gradient instability and poor sample loading capacity associated with carrier ampholyte–generated pH gradients

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Immunoassay Test for a substance by its reactivity with an antibody

Immunoblotting Blot detection by antibody binding

Immunodetection Detection of a molecule by its binding to an antibody

Immunoglobulin Antibody; protein produced in response to an antigen, which specifically binds the portion of the antigen that initiated its production

Ion front Group of ions moving together during electrophoresis, marking the movement of the buffer from the upper buffer reservoir. Due to their small size, they are not hindered by a sieving matrix and move together primarily because of their charge

Ionic strength Measure of the ionic concentration of a solution that affects its resistance

Isoelectric focusing (IEF) Electrophoresis technique that separates proteins according to their isoelectric point (pI)

Isoelectric point (pI) pH value at which a molecule carries no electrical charge, or at which the negative and positive charges are equal

Ligand Molecule that binds another in a complex

Monomer Unit that makes up a polymer (acrylamide is a monomer that is polymerized into polyacrylamide)

Mini-PROTEAN® Cells and Gels Family of Bio-Rad products used for mini-format vertical electrophoresis; includes the Mini-PROTEAN Tetra and Mini-PROTEAN® 3 Dodeca™ Cells, and Mini-PROTEAN Precast Gels

Native PAGE Version of PAGE that retains native protein configuration, performed in the absence of SDS and other denaturing agents

Ohm's Law Describes the mutual dependence of three electrical parameters (V, volts; I, ampere; R, ohm): V = I x R

PAGE Polyacrylamide gel electrophoresis, a common method of separating proteins

Polyacrylamide Anticonvective sieving matrix used in gel electrophoresis. Polyacrylamide gels are cast using mixtures of acrylamide monomers with a cross-linking reagent, usually N,N'-methylenebisacrylamide (bis), both solubilized in buffer

Polyacrylamide gel electrophoresis (PAGE) Electrophoresis technique that uses polyacrylamide as the separation medium

PowerPac™ Power Supplies Family of Bio-Rad power supplies

Power supply Instrument that provides the electric power to drive electrophoresis and electrophoretic blotting experiments

Precision Plus Protein™ Standards Bio-Rad's family of recombinant protein standards

Preparative electrophoresis Electrophoresis techniques that separate large volumes of protein samples (nanogram to gram quantities of protein), generally for the purposes of purification or fractionation (to reduce sample complexity)

Primary antibody Antibody that binds a molecule of interest

PROTEAN® Cells Family of Bio-Rad products used for large-format vertical electrophoresis; includes the PROTEAN II xi, PROTEAN II XL, and PROTEAN Plus® Dodeca™ Cells

Protein standards Mixtures of well-characterized or recombinant proteins used to monitor separation and estimate the size and concentration of the proteins separated in a gel

Prestained standards Mixture of molecular weight marker proteins that have covalently attached dye molecules, which render the bands visible during electrophoresis and transfer

***RC DC*™ Assay Kit** Bio-Rad's reductant- and detergent-compatible protein assay kit

Resolving gel Portion of a discontinuous electrophoresis gel that separates the different bands from each other

R_f value Relative distance a protein has traveled compared to the distance traveled by the ion front. This value is used to compare proteins in different lanes and even in different gels. It can be used with standards to generate standard curves, from which the molecular weight or isoelectric point of an unknown may be determined

Running buffer Buffer that provides the ions for the electrical current in an electrophoresis run. It may also contain denaturing agents. The running buffer provides the trailing ions in discontinuous electrophoresis

Sample buffer Buffer in which a sample is suspended prior to loading onto a gel. SDS-PAGE sample buffer typically contains denaturing agents (including reducing agents and SDS), tracking dye, and glycerol

SDS-PAGE Separation of molecules by molecular weight in a polyacrylamide gel matrix in the presence of a denaturing detergent, sodium dodecyl sulfate (SDS). SDS denatures polypeptides and binds to proteins at a constant charge-to-mass-ratio. In a sieving polyacrylamide gel, the rate at which the resulting SDS-coated proteins migrate in the gel is relative only to their size and not to their charge or shape

Secondary antibody Reporter antibody that binds to a primary antibody; used to facilitate detection

Sodium dodecyl sulfate (SDS) Anionic detergent that denatures proteins and binds to polypeptides in a constant weight ratio of 1:4 (SDS:polypeptide)

Spacers Small blocks set between the two glass plates at the sides of a gel cassette, which create a space between the glass plates in which to pour the slab gel monomer solution

Stacking gel Portion of a discontinuous electrophoresis gel that concentrates the components of the sample to create a very thin starting zone; bands are then separated from each other in the resolving gel

Stain-free technology Protein detection technology involving UV-induced haloalkane modification of protein tryptophan residues. Continued exposure to UV light causes fluorescence of the modified proteins, which are then detected by a CCD imager. Sensitivity of this technique is generally equal to or better than Coomassie staining

Stained standards Mixture of molecular weight marker proteins that have covalently attached dye molecules; the bands are visible during electrophoresis and transfer

Standard Collection of molecules with known properties, such as molecular weight or isoelectric point. Often used to create standard curves, from which properties of an unknown may be determined

TEMED Used with APS (initiator) to catalyze the polymerization of acrylamide and bisacrylamide in making a polyacrylamide gel; N,N,N',N'-tetramethylethylenediamine

TGX™ Gels Bio-Rad's Tris-glycine extended shelf life precast gels

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Total protein stain	Reagent that binds nonspecifically to proteins; used to detect the entire protein pattern on a blot or gel
Total protein normalization	In total protein normalization, the abundance of the target protein is normalized to the total amount of protein in each lane, removing variations associated with normalization against a single protein
Tricine	Organic compound used in SDS-PAGE as a buffer component to replace glycine and improve resolution of small (down to 1–5 kD) proteins
Tris	Organic component of buffer solutions that has an effective pH range of 7.0–9.2; tris(hydroxymethyl) aminomethane
Transfer	Immobilization of proteins or other molecules onto a membrane by electrophoretic or passive means
Triton X-100	Nonionic detergent widely used for protein solubilization (for IEF and 2-D electrophoresis)
Tween 20	Nonionic detergent; used in blot detection procedures as a blocking reagent or in wash buffers to minimize nonspecific binding and background
Unstained standards	Mixture of molecular weight marker proteins that do not have covalently attached dye molecules; the bands are invisible during electrophoresis and transfer, but are useful for molecular weight determination
Urea	Chaotrope usually included at rather high concentrations (9.5 M) in sample solubilization buffers for denaturing IEF and 2-D PAGE
Western blotting	Immobilization of proteins onto a membrane and subsequent detection by protein-specific binding and detection reagents
Zymogram PAGE	Electrophoresis technique used to detect and characterize collagenases and other proteases within the gel. Gels are cast with gelatin or casein, which acts as a substrate for the enzymes that are separated in the gel under nonreducing conditions

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Bio-Rad Bulletins

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2651	2-D Electrophoresis for Proteomics: A Methods and Product Manual
2895	Protein Blotting Guide, A Guide to Transfer and Detection
1156	Acrylamide Polymerization – a Practical Approach
1909	Modification of Bio-Rad *DC*™ Protein Assay for Use with Thiols
6001	Rapid Validation of Purified Proteins Using Criterion™ Stain Free™ Gels
5910	Mini-PROTEAN® TGX™ Precast Gel: A Gel for SDS-PAGE with Improved Stability — Comparison with Standard Laemmli Gels
5911	Mini-PROTEAN TGX Precast Gel: A Versatile and Robust Laemmli-Like Precast Gel for SDS-PAGE
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Product Information

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1069	Colorimetric Protein Assays
2414	The Little Book of Standards
2998	Protein Standards Application Guide
2317	Ready-to-Run Buffers and Solutions Brochure
5535	Mini-PROTEAN® Tetra Cell Brochure
5871	Mini-PROTEAN® TGX™ Precast Gels Product Information Sheet
2710	Criterion™ Precast Gel System Brochure
2911	Criterion XT Precast Gels Product Information Sheet
5974	Criterion TGX Stain-Free Precast Gels Product Information Sheet
1760	PROTEAN® II xi and XL Cells Product Information Sheet
6371	Electrophoresis Power Supplies Brochure
2423	Bio-Safe™ Coomassie Stain Brochure
5346	Flamingo™ Fluorescent Gel Stain Product Information Sheet
5900	Oriole™ Fluorescent Gel Stain Product Information Sheet
3096	Expression Proteomics Brochure

Instruction Manuals

Bulletin	Title
10007296	Mini-PROTEAN Tetra Cell
1658100	Mini-PROTEAN Precast Gels
4006183	Criterion Cell
4006213	PowerPac Basic Power Supply
4006222	PowerPac HC Power Supply
4006223	PowerPac Universal Power Supply
4110001	Criterion Gel Application Guide
4110130	Criterion XT Precast Gels
4110065	Quick Start™ Bradford Protein Assay
4110107	*RC DC*™ Protein Assay
4000126-14	The Discovery Series: Quantity One® 1-D Analysis Software
LIT33	Bio-Rad Protein Assay
LIT448	*DC* Protein Assay

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Ordering Information

Electrophoresis Instrumentation

Catalog #	Description
Mini-PROTEAN® Tetra Cells and Systems	
1658000	**Mini-PROTEAN Tetra Cell**, 10-well, 0.75 mm thickness; 4-gel system includes 5 combs, 5 sets of glass plates, 2 casting stands, 4 casting frames, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam
1658001	**Mini-PROTEAN Tetra Cell**, 10-well, 1.0 mm thickness; 4-gel system includes 5 combs, 5 sets of glass plates, 2 casting stands, 4 casting frames, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam
1658002	**Mini-PROTEAN Tetra Cell**, 2 10-well, 0.75 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam
1658003	**Mini-PROTEAN Tetra Cell**, 10-well, 1.0 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam
1658004	**Mini-PROTEAN Tetra Cell for Mini Precast Gels**, 4-gel system includes electrode assembly, clamping frame, companion module, tank, lid with power cables, mini cell buffer dam
1658005	**Mini-PROTEAN Tetra Cell for Mini Precast Gels**, 2-gel system includes electrode assembly, clamping frame, tank, lid with power cables, mini cell buffer dam
1658006	**Mini-PROTEAN Tetra Cell**, 10-well, 1.5 mm thickness; 4-gel system includes 5 combs, 5 sets of glass plates, 2 casting stands, 4 casting frames, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam
1658007	**Mini-PROTEAN Tetra Cell**, 10-well, 1.5 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam
1658025	**Mini-PROTEAN Tetra Cell and PowerPac™ Basic Power Supply**, includes 1658001 and 1645050
1658026	**Mini-PROTEAN Tetra Cell and PowerPac™ Universal Power Supply**, includes 1658001 and 1645070
1658027	**Mini-PROTEAN Tetra Cell and PowerPac™ HC Power Supply**, includes 1658001 and 1645052
1658028	**Mini-PROTEAN Tetra Cell and PowerPac™ HV Power Supply**, includes 1658001 and 1645056
1658029	**Mini-PROTEAN Tetra Cell and Mini Trans-Blot® Module**, includes 1658001 and 1703935
1658030	**Mini-PROTEAN Tetra Cell for Mini Precast Gels and Mini Trans-Blot Module**, includes 1658004 and 1703935
1658033	**Mini-PROTEAN Tetra Cell, Mini Trans-Blot Module, and PowerPac Basic Power Supply**, includes 165-8001, 170-3935, and 164-5050
1658034	**Mini-PROTEAN Tetra Cell for Mini Precast Gels, Mini Trans-Blot Module, and PowerPac Basic Power Supply**, includes 1658004, 1703935, and 1645050
1658035	**Mini-PROTEAN Tetra Cell, Mini Trans-Blot Module, and PowerPac HC Power Supply**, includes 1658001, 1703935, and 1645052
1658036	**Mini-PROTEAN Tetra Cell for Mini Precast Gels**, Mini Trans-Blot Module, and PowerPac HC Power Supply, includes 1658004, 1703935, and 1645052
Mini-PROTEAN® Dodeca™ Cells and Systems	
1654100	**Mini-PROTEAN 3 Dodeca Cell**, includes electrophoresis tank with built-in cooling coil, lid with power cables, 6 electrophoresis clamping frames, 2 buffer dams, drain line, 2 gel releasers
1654101	**Mini-PROTEAN 3 Dodeca Cell with Multi-Casting Chamber**, same as 165-4100 with multi-casting chamber, 15 separation sheets, 8 acrylic blocks, tapered luer connector, stopcock valve
Criterion™ Cells and Systems	
1656001	**Criterion Cell**, includes buffer tank, lid with power cables, 3 sample loading guides (12 + 2-well, 18-well, 26-well)
1656019	**Criterion Cell and PowerPac Basic Power Supply**, 100–120/220–240 V, includes 1656001 and 1645050
Criterion™ Dodeca™ Cells and Systems	
1654130	**Criterion Dodeca Cell**, includes electrophoresis buffer tank with built-in cooling coil, lid with power cables
1654138	**Criterion Dodeca Cell and PowerPac HC Power Supply**, includes 1654130 and 1645052
1654139	**Criterion Dodeca Cell and PowerPac Universal Power Supply**, includes 1654130 and 1645070
1655133	**Criterion Dodeca Cell and 6-Row AnyGel™ Stand**, includes 1654130 and 1655131
PROTEAN® II xi Cells	
1651801	**PROTEAN II xi Cell**, 16 cm, without spacers and combs
1651802	**PROTEAN II xi Cell**, 16 cm, 1.5 mm spacers (4), 15-well combs (2)
1651803	**PROTEAN II xi Cell**, 16 cm, 1.0 mm spacers (4), 15-well combs (2)
1651804	**PROTEAN II xi Cell**, 16 cm, 0.75 mm spacers (4), 15-well combs (2)
1651811	**PROTEAN II xi Cell**, 20 cm, without spacers and combs
1651812	**PROTEAN II xi Cell**, 20 cm, 1.5 mm spacers (4), 15-well combs (2)
1651813	**PROTEAN II xi Cell**, 20 cm, 1.0 mm spacers (4), 15-well combs (2)
1651814	**PROTEAN II xi Cell**, 20 cm, 0.75 mm spacers (4), 15-well combs (2)
PROTEAN® Plus Dodeca™ Cells and Systems	
1654150	**PROTEAN Plus Dodeca Cell**, 100/120 V, includes electrophoresis buffer tank with built-in ceramic cooling core, lid, buffer recirculation pump with tubing, 2 gel releasers
1654140	**PROTEAN Plus Dodeca Cell (100/120 V) and PowerPac HC Power Supply**, includes 1654150 and 1645052
1654142	**PROTEAN Plus Dodeca Cell (100/120 V) and PowerPac Universal Power Supply**, includes 1654150 and 1645070
1654144	**PROTEAN Plus Dodeca Cell (100/120 V)**, Trans-Blot Plus Cell, and PowerPac Universal Power Supply, includes 1654150, 1703990, and 1645070
1655134	**PROTEAN Plus Dodeca Cell (100/120 V) and Two 6-Row AnyGel Stands**, includes 1654150 and two 1655131
1654151	**PROTEAN Plus Dodeca Cell**, 220/240 V, includes electrophoresis buffer tank with built-in ceramic cooling core, lid, buffer recirculation pump with tubing, 2 gel releasers
1654141	**PROTEAN Plus Dodeca Cell (220/240 V) and PowerPac HC Power Supply**, includes 1654151 and 1645052
1654143	**PROTEAN Plus Dodeca Cell (220/240 V) and PowerPac Universal Power Supply**, includes 1654151 and 1645070
1654145	**PROTEAN Plus Dodeca Cell (220/240 V)**, Trans-Blot Plus Cell, and PowerPac Universal Power Supply, includes 1654151, 1703990, and 1645070
1655135	**PROTEAN Plus Dodeca Cell (220/240 V) and Two 6-Row AnyGel Stands**, includes 1654151 and two 1655131
Power Supplies	
1645050	**PowerPac Basic Power Supply**, 100–120/220–240 V
1645052	**PowerPac HC Power Supply**, 100–120/220–240 V
1645056	**PowerPac HV Power Supply**, 100–120/220–240 V
1645070	**PowerPac Universal Power Supply**, 100–120/220–240 V
Sample Preparation Kits	
1632141	**MicroRotofor™ Cell Lysis Kit (Mammal)**, 15 preps, includes 50 ml protein solubilization buffer (PSB), ReadyPrep™ mini grinders (2 packs of 10 each)
1632142	**MicroRotofor Cell Lysis Kit (Plant)**, 10 preps, includes 50 ml protein solubilization buffer (PSB), ReadyPrep 2-D cleanup kit (50 reaction size)
1632143	**MicroRotofor Cell Lysis Kit (Yeast)**, 15 preps, includes 50 ml protein solubilization buffer (PSB), 15 ml yeast suspension buffer, 2 x 0.5 ml lyticase (1.5 U/µl)
1632144	**MicroRotofor Cell Lysis Kit (Bacteria)**, 15 preps, includes 50 ml protein solubilization buffer (PSB), 25 ml bacteria suspension buffer, 1 ml lysozyme (1,500 U/µl)
1632140	**ReadyPrep 2-D Cleanup Kit**, 5 preps
7326221	**Micro Bio-Spin™ 6 Columns**, includes 25 columns in Tris buffer, 50 collection tubes
7326227	**Bio-Spin® 6 Columns**, includes 25 columns in Tris buffer, 50 collection tubes
7326228	**Bio-Spin 6 Columns**, includes 100 columns in Tris buffer, 200 collection tubes
Protein Assay Kits and Instruments	
5000001	**Bio-Rad Protein Assay Kit I**, includes 450 ml dye reagent concentrate, bovine β-globulin standard; sufficient for 440 standard assays or 2,200 microplate assays
5000002	**Bio-Rad Protein Assay Kit II**, includes 450 ml dye reagent concentrate, bovine serum albumin standard; sufficient for 440 standard assays or 2,200 microplate assays
5000111	***DC*™ Protein Assay Kit I**, includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent, 5 ml surfactant solution, bovine β-globulin standard; sufficient for 450 standard assays
5000112	***DC* Protein Assay Kit II**, includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent, 5 ml surfactant solution, bovine serum albumin standard; sufficient for 450 standard assays
5000120	***RC DC*™ Protein Assay Reagents Package**, includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent, 5 ml surfactant solution; sufficient for 450 standard assays
5000121	***RC DC* Protein Assay Kit I**, includes RC reagents package, DC reagents package, bovine β-globulin standard; sufficient for 450 standard assays
5000122	***RC DC* Protein Assay Kit II**, includes RC reagents package, DC reagents package, bovine serum albumin standard; sufficient for 450 standard assays
5000201	**Quick Start™ Bradford Protein Assay Kit 1**, includes 1x dye reagent (1 L), bovine serum albumin standard (5 x 2 mg/ml); sufficient for 200 standard assays or 4,000 microplate assays
5000202	**Quick Start Bradford Protein Assay Kit 2**, includes 1x dye reagent (1 L), bovine serum albumin standard set (2 sets of 7 concentration standards, 0.125–2.0 mg/ml, 2 ml)
5000203	**Quick Start Bradford Protein Assay Kit 3**, includes 1x dye reagent (1 L), bovine β-globulin standard (5 x 2 mg/ml)
5000204	**Quick Start Bradford Protein Assay Kit 4**, includes 1x dye reagent (1 L), bovine β-globulin standard set (2 sets of 7 concentration standards, 0.125–2.0 mg/ml, 2 ml)
1702502	**Standard Cuvette**, 1–3.5 ml, quartz
1702511	**trUView™ Cuvettes**, pack of 100, individually packaged, disposable DNase- and RNase-free cuvettes
Sample Preparation Buffers and Reagents	
1610747	**4x Laemmli Sample Buffer**, 10 ml
1610737	**Laemmli Sample Buffer**, 30 ml
1610738	**Native Sample Buffer**, 30 ml
1610739	**Tricine Sample Buffer**, 30 ml
1610763	**IEF Sample Buffer**, 30 ml
1610791	**XT Sample Buffer**, 4x, 10 ml
1610792	**XR Reducing Agent**, 1 ml
1610719	**Tris**, 1 kg
1610718	**Glycine**, 1 kg
1610301	**SDS**, 100 g
1610416	**SDS Solution**, 10% (w/v), 250 ml
1662404	**10% Tween 20**, 5 ml
1610710	**2-Mercaptoethanol**, 25 ml
1610611	**Dithiothreitol**, 5 g
1610404	**Bromophenol Blue**, 10 g
1610730	**Urea**, 250 g

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Catalog #	Description
Protein Standards	
Recombinant Prestained Protein Standards	
1610393	**Precision Plus Protein All Blue Standards Value Pack**, 5 x 500 µl
1610373	**Precision Plus Protein All Blue Standards**, 500 µl
1610394	**Precision Plus Protein Dual Color Standards Value Pack**, 5 x 500 µl
1610374	**Precision Plus Protein Dual Color Standards**, 500 µl
1610397	**Precision Plus Protein Dual Xtra Standards Value Pack**, 5 x 500 µl
1610377	**Precision Plus Protein Dual Xtra Standards**, 500 µl
1610395	**Precision Plus Protein Kaleidoscope Standards Value Pack**, 5 x 500 µl
1610375	**Precision Plus Protein Kaleidoscope Standards**, 500 µl
1610399	**Precision Plus Protein WesternC Standards Value Pack**, 5 x 250 µl
1610376	**Precision Plus Protein WesternC Standards**, 250 µl
1610398	**Precision Plus Protein WesternC (Standards + HRP) Value Pack**, 5 x 250 µl
1610385	**Precision Plus Protein WesternC (Standards + HRP)**, 250 µl
Recombinant Unstained Protein Standards	
1610396	**Precision Plus Protein Unstained Standards Value Pack**, 5 x 1000 µl
1610363	**Precision Plus Protein Unstained Standards**, 1000 µl

Catalog #	Description
Running Buffers and Reagents	
1610732	**10x Tris/Glycine/SDS**, 1 L
1610734	**10x Tris/Glycine**, 1 L
1610744	**10x Tris/Tricine/SDS**, 1 L
1610788	**XT MOPS Running Buffer**, 20x, 500 ml
1610789	**XT MES Running Buffer**, 20x, 500 ml
1610790	**XT Tricine Running Buffer**, 20x, 500 ml
1610793	**XT MOPS Buffer Kit**, includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20x XT reducing agent
1610796	**XT MES Buffer Kit**, includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20x XT reducing agent
1610797	**XT Tricine Buffer Kit**, includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20x XT reducing agent
1610761	**10x IEF Anode Buffer**, 250 ml
1610762	**10x IEF Cathode Buffer**, 250 ml
1610765	**Zymogram Renaturation Buffer**, 125 ml
1610766	**Zymogram Development Buffer**, 125 ml
1610729	**EDTA**, 500 g
1610718	**Glycine**, 1 kg
1610713	**Tricine**, 500 g
1610719	**Tris**, 1 kg
Gel Casting Buffers and Reagents	
1615100	**SDS-PAGE Reagent Starter Kit**, includes 100 g acrylamide, 5 g bis, 5 ml TEMED, 10 g ammonium persulfate
1610100	**Acrylamide**, 99.9%, 100 g
1610120	**Acrylamide/Bis Powder**, 19:1, 30 g
1610122	**Acrylamide/Bis Powder**, 37.5:1, 30 g
1610140	**40% Acrylamide Solution**, 500 ml
1610144	**40% Acrylamide/Bis Solution**, 19:1, 500 ml
1610146	**40% Acrylamide/Bis Solution**, 29:1, 500 ml
1610148	**40% Acrylamide/Bis Solution**, 37.5:1, 500 ml
1610154	**30% Acrylamide/Bis Solution**, 19:1, 500 ml
1610156	**30% Acrylamide/Bis Solution**, 29:1, 500 ml
1610158	**30% Acrylamide/Bis Solution**, 37.5:1, 500 ml
1610200	**Bis Crosslinker**, 5 g
1610800	**TEMED**, 5 ml
1610798	**Resolving Gel Buffer**, 1.5 M tris-HCl, pH 8.8, 1 L
1610700	**Ammonium Persulfate (APS)**, 10 g
1610799	**Stacking Gel Buffer**, 0.5 M tris-HCl, pH 6.8, 1 L

Precast Gels

Description					
10-Gels/Box	**10-Well 30 µl**	**10-Well 50 µl**	**15-Well 15 µl**	**IPG Well 7cm IPG Strip**	**12-Well 20 µl**
Mini-PROTEAN® TGX™ Resolving Gels					
7.5%	4561023	4561024	4561026	4561021	4561025
10%	4561033	4561034	4561036	4561031	4561035
12%	4561043	4561044	4561046	4561041	4561045
18%	4561073	4561074	4561076	4561071	4561075
4–15%	4561083	4561084	4561086	4561081	4561085
4–20%	4561093	4561094	4561096	4561091	4561095
8–16%	4561103	4561104	4561106	4561101	4561105
10–20%	4561113	4561114	4561116	4561111	4561115
Any kD	4569033	4569034	4569036	4569031	4569035
Mini-PROTEAN® TGX Stain-Free™ Gels					
7.5%	4568023	4568024	4568026	4568021	4568025
10%	4568033	4568034	4568036	4568031	4568035
12%	4568043	4568044	4568046	4568041	4568045
4–15%	4568083	4568084	4568086	4568081	4568085
4–20%	4568093	4568094	4568096	4568091	4568095
8–16%	4568103	4568104	4568106	4568101	4568105
Any kD	4568123	4568124	4568126	4568121	4568125
Mini-PROTEAN Tris-Tricine Gels (Pack of 2)					
16.5% Resolving Gel	4563063	4563064	4563066	—	4563065*
10–20% Resolving Gel	4563113	4563114	4563116*	—	4563115*
Mini-PROTEAN TBE Gels (Pack of 2)					
5% TBE Gel	4565013	4565014*	4565016	—	4565015*
Mini-PROTEAN TBE-Urea Gels (Pack of 2)					
10% TBE-Urea Gel	4566033*	—	4566036*	—	—
15% TBE-Urea Gel	4566053*	—	4566056*	—	4566055*

All formats are available as both ten packs (catalog numbers listed) and two packs. To order as a two pack, add an “S” to the end of the catalog number for the corresponding ten pack.

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Description	12+2-Well** 45 µl	18-Well 30 µl	26-Well* 15 µl	Prep+2-Well** 800 µl	IPG+1-Well** 11 cm IPG Strip
Criterion™ TGX™ Gels**					
7.5%	5671023	5671024	5671025	—	—
10%	5671033	5671034	5671035	—	—
12%	5671043	5671044	5671045	—	—
4–15%	5671083	5671084	5671085	5671082	5671081
4–20%	5671093	5671094	5671095	5671092	5671091
8–16%	5671103	5671104	5671105	5671102	5671101
Any kD	5671123	5671124	5671125	5671122	5671121
Criterion™ TGX Stain-Free™ Gels**					
7.5%	5678023	5678024	5678025	—	—
12%	5678043	5678044	5678045	—	—
18%	5678073	5678074	5678075	5678072	5678071
4–15%	5678083	5678084	5678085	5678082	5678081
4–20%	5678093	5678094	5678095	5678092	5678091
8–16%	5678103	5678104	5678105	5678102	5678101
Any kD	5678123	5678124	5678125	5678122	5678121
Criterion XT Bis-Tris Gels***					
10% Resolving Gel	3450111	3450112	3450113	—	3450115
12% Resolving Gel	3450117	3450118	3450119	3450120†	3450121
4–12% Resolving Gel	3450123	3450124	3450125	3450126†	3450127
Criterion XT Tris-Acetate Gels					
3–8% Resolving Gel	3450129	3450130	3450131	—	—
Criterion Tris-HCl Gels					
10% Resolving Gel	3450009	3450010	3450011	—	3450101
12.5% Resolving Gel	3450014	3450015	3450016	—	3450102
15% Resolving Gel	3450019	3450020	3450021	—	—
4–15% Linear Gradient	3450027	3450028	3450029	—	3450103
4–20% Linear Gradient	3450032	3450033	3450034	—	3450104
10–20% Linear Gradient	3450042	3450043	3450044	—	3450107
Criterion Tris-Tricine Gels					
16.5% Tris-Tricine	3450063	3450064	3450065†	—	—
Criterion IEF Gels					
pH 3–10	3450071†	3450072†	3450073†	—	—
pH 5–8	—	3450076†	—	—	—

* Multichannel pipet compatible.

** Includes reference well(s).

*** Purchase of this product is accompanied by a limited license under U.S. Patent Numbers 6,143,154; 6,096,182; 6,059,948; 5,578,180; 5,922,185; 6,162,338; and 6,783,651 and corresponding foreign patents.

† Please allow up to 2 weeks for delivery.

Gel Casting Accessories

See catalog or bio-rad.com for a complete listing of accessories, including available empty gel cassettes and glass plates, spacers, combs, etc.

Catalog #	Description
1655131	**AnyGel Stand, 6-row**, holds 6 PROTEAN® Gels or 12 Criterion Gels
1654131	**AnyGel Stand, single-row**, holds 1 PROTEAN Gel or 2 Criterion Gels

Total Protein Gel Stains

1610803	**QC Colloidal Coomassie Solution Kit**, 1 L, ready-to-use, non-hazardous colloidal Coomassie G-250 Stain for protein polyacrylamide gels
1610786	**Bio-Safe™ Coomassie Stain**, 1 L
1610787	**Bio-Safe Coomassie Stain**, 5 L
1610435	**Coomassie Brilliant Blue R-250 Staining Solutions Kit**, includes 1 L Coomassie Brilliant Blue R-250 Staining Solution, 2 x 1 L Coomassie Brilliant Blue R-250 Destaining Solution
1610436	**Coomassie Brilliant Blue R-250 Staining Solution**, 1 L
1610438	**Coomassie Brilliant Blue R-250 Destaining Solution**, 1 L
1610400	**Coomassie Brilliant Blue R-250**, 10 g
1610406	**Coomassie Brilliant Blue G-250**, 10 g
1610443	**Silver Stain Kit**, includes oxidizer concentrate, silver reagent concentrate, silver stain developer, stains 20 full size or 48 mini gels
1610449	**Silver Stain Plus™ Kit**, includes fixative enhancer concentrate, silver complex solution, reduction moderator solution, image development reagent, development accelerator reagent, stains 13 full size or 40 mini gels
1610496	**Oriole™ Fluorescent Gel Stain**, 1x solution, 1 L
1610492	**Flamingo™ Fluorescent Gel Stain**, 10x solution, 500 ml
1703125	**SYPRO Ruby Protein Gel Stain**, 1x solution, 1 L
1610440	**Zinc Stain and Destain Kit**, includes 125 ml of 10x zinc stain solution A, 125 ml of 10x zinc stain solution B, 125 ml of 10x zinc destain solution
1610470	**Copper Stain and Destain Kit**, includes 125 ml of 10x copper stain, 125 ml of 10x copper destain solution

High-Throughput Stainers

1653400	**Dodeca™ Stainer, large**, 100–240 V, includes 13 trays (12 clear, 1 white), 12 tray attachments, shaking rack, solution tank, lid with shaker motor, shaker control unit, gel clip
1653401	**Dodeca Stainer, small**, 100–240 V, includes 13 trays (12 clear, 1 white), 12 Criterion tray attachments, shaking rack, solution tank, lid with shaker motor, shaker control unit, gel clip

Catalog #	Description
Imaging Systems	
12003153	**ChemiDoc™ Imaging System,** gel and blot imaging and analysis system, includes internal computer, 12" touch-screen display, camera, Image Lab™ Touch Software, Image Lab Software, Blot/UV/Stain-Free Sample Tray. Optional upgrade path to ChemiDoc MP for fluorescence detection
12003154	**ChemiDoc MP Imaging System,** gel and blot imaging and analysis system, includes internal computer, 12" touch-screen display, camera, Image Lab Touch Software, Image Lab Software, Blot/UV/Stain-Free Sample Tray
1707991	**GS-900™ Calibrated Densitometry System**, gel densitometry system, PC compatible, includes scanner, cables, Image Lab Software, optional 21 CFR Part 11 and Installation Qualification/ Operations Qualification
1708195	**Gel Doc™ XR+ System with Image Lab Software**, PC or Mac, includes darkroom, UV transilluminator, epi-white illumination, camera, cables, Image Lab Software
1708270	**Gel Doc EZ System with Image Lab Software**, PC or Mac, includes darkroom, camera, cables, Image Lab software; samples trays (#1708271, 1708272, 1708273, or 1708274) are sold separately; sample trays are required to use the system
1708265	**ChemiDoc™ XRS+ System with Image Lab Software**, PC or Mac, includes darkroom, UV transilluminator, epi-white illumination, camera, power supply, cables, Image Lab Software
1709400	**Personal Molecular Imager™ (PMI) System**, PC or Mac, 110/240 V, includes sample tray set and USB2 cable
Analysis Software	
1709690	**Image Lab Software**

Purchase of Criterion XT Bis-Tris gels, XT MOPS running buffer, XT MES running buffer, XT MOPS buffer kit, and XT MES buffer kit is accompanied by a limited license under U.S. Patent Numbers 6,143,154; 6,096,182; 6,059,948; 5,578,180; 5,922,185; 6,162,338; and 6,783,651, and corresponding foreign patents.

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Precision Plus Protein standards are sold under license from Life Technologies Corporation, Carlsbad, CA, for use only by the buyer of the product. The buyer is not authorized to sell or resell this product or its components.

StrepTactin is covered by German patent application P 19641876.3. Bio-Rad Laboratories, Inc. is licensed by Institut für Bioanalytik GmbH to sell these products for research use only.

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BIO-RAD

Bio-Rad Laboratories, Inc.

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