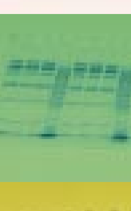
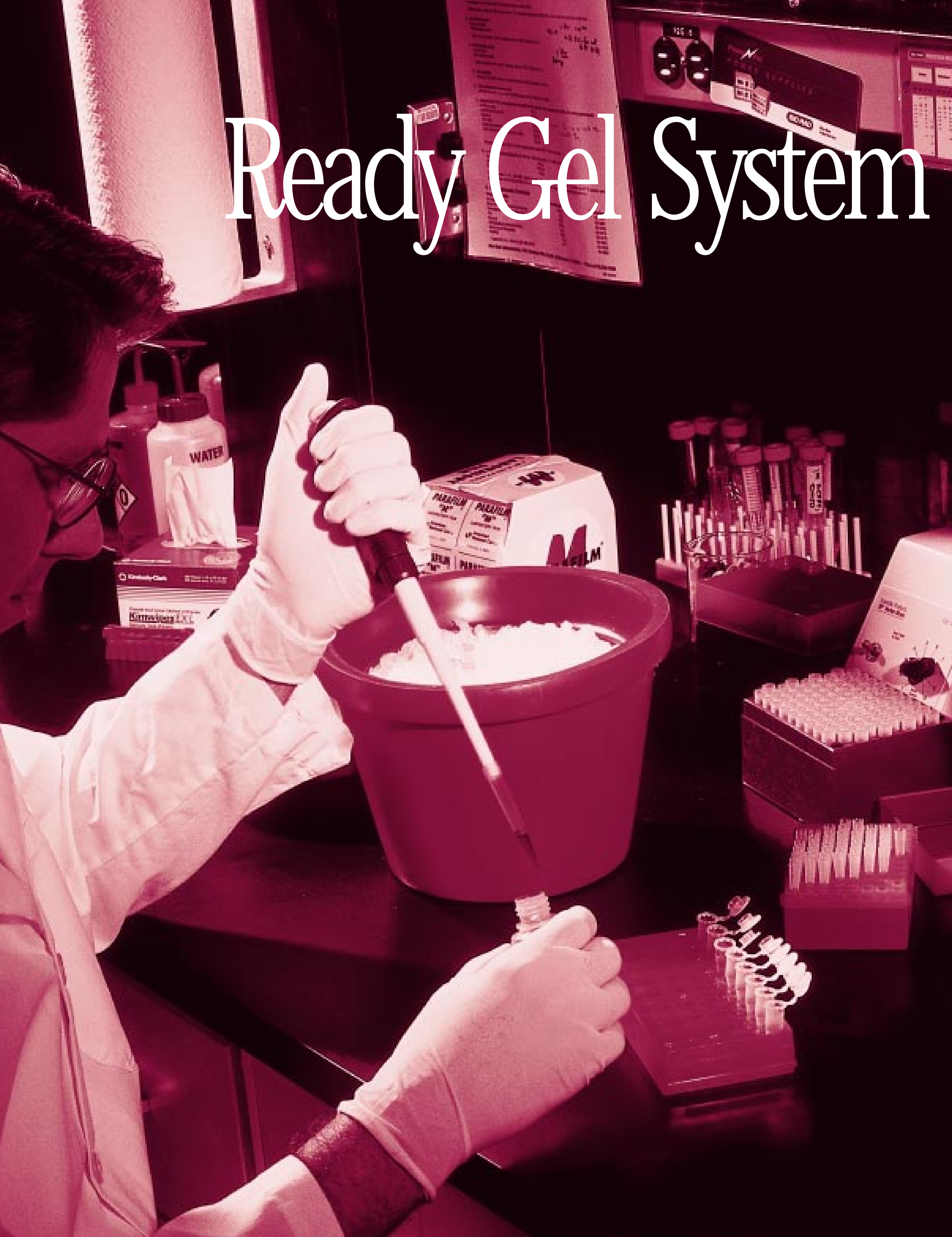
Ready Gel System RESOURCE GUIDE



Ready Gel System



Resource Guide

Exploring, discovering, and understanding the world around us - the biological systems interacting with the environment - is a process that fascinates us all. Research uncovers more information each day, and we are realizing the benefits in numerous new ways.

Now more than ever, time is a critical factor in biomolecular research, and Bio-Rad is working diligently to produce reagents and instruments that help you make the most of it. The tools we develop reduce setup time and help you find answers that much faster.

One of the most trusted, efficient, and powerful resolving techniques used today is electrophoresis. Bio-Rad has a long history of success in this area, offering innovative products as well as the experience and expertise necessary to make this technique work for you.

With this in mind, we present the Ready Gel System Resource Guide. It should serve you well as both a guide to Bio-Rad vertical electrophoresis equipment and reagents, and as a useful reference for general electrophoresis protocols and information.

Read on, and remember that by building your lab with electrophoresis products from Bio-Rad, you will indeed ***Accelerate Your Research!***

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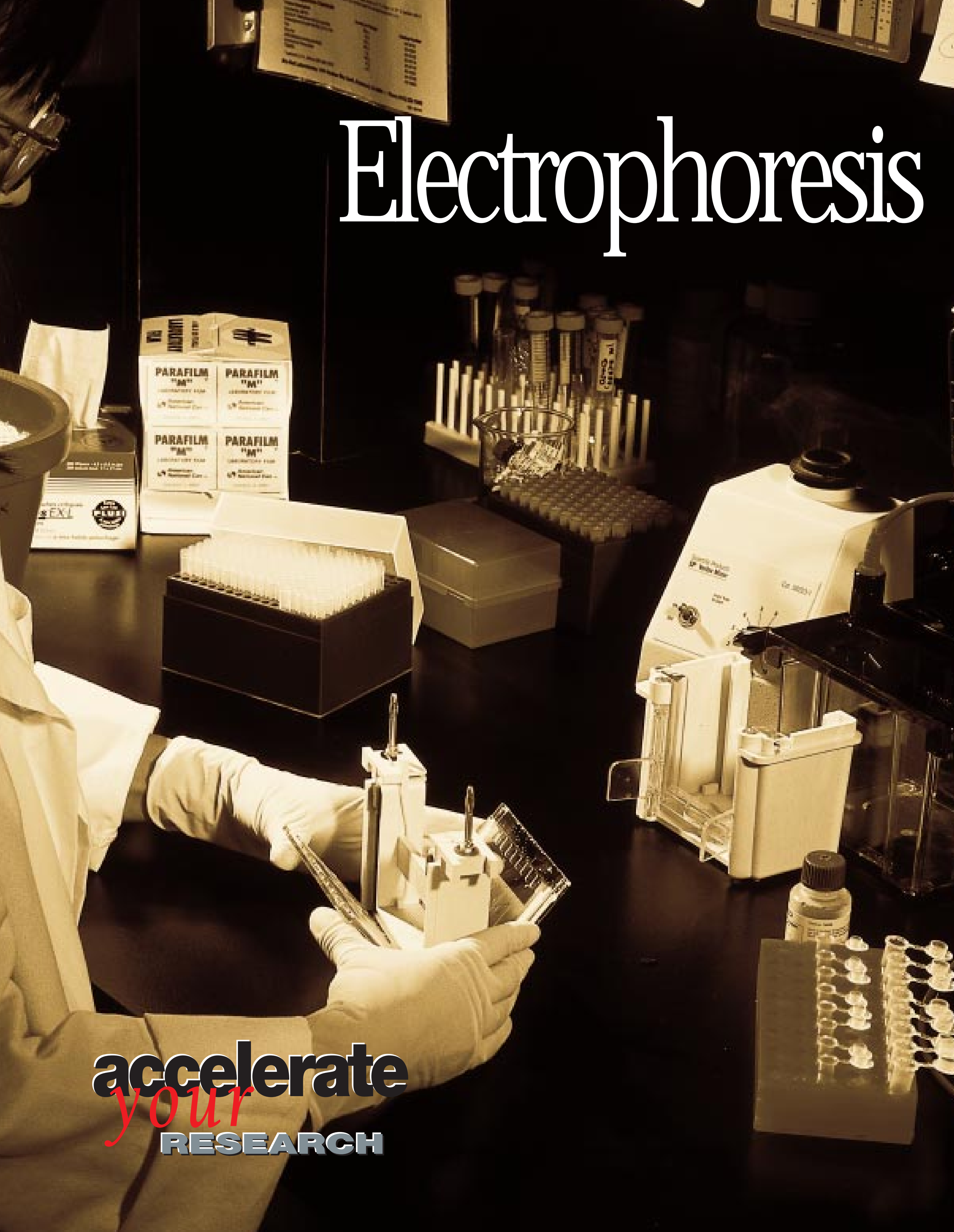
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Theory

Polyacrylamide Gel Electrophoresis

Gel electrophoresis is one of the most frequently used and most powerful techniques in laboratory research. This well known method separates biomolecules in complex mixtures according to their physical properties of size and charge. All the components of the electrophoresis system are now available on a ready-to-use basis, including precast Ready Gels, premixed running and sample buffers, and premixed staining solutions, making this technique easier than ever to access.

It is helpful to have a basic understanding of electrophoresis concepts so that when a new sample is being assessed, a logical approach can be taken in selecting the proper tools. This section provides some basic theoretical aspects of gel electrophoresis. The remainder of this guide provides information on the Bio-Rad products available to support your electrophoresis efforts.

During electrophoresis, there is an intricate interaction of samples, gel matrix, buffers, and electricity resulting in separate bands of individual molecules. Applications for electrophoresis are very broad, extending into both protein, nucleic acid, and carbohydrate work. Protein electrophoresis is generally performed in polyacrylamide gels, while nucleic acid electrophoresis generally uses agarose gels, although TBE polyacrylamide gels are common for resolving DNA fragments of 50 - 2,000 bp sizes.

The focus of this guide is on separations using acrylamide. Polyacrylamide gels are composed of long linear polyacrylamide chains crosslinked with bis-acrylamide (bis) to create a network of pores interspersed between bundles of polymer. The structural features of a gel can be thought of as a three-dimensional sieve, made up of random distributions of solid material and pores. The ability of proteins or nucleic acids to move through the gel depends on their size and structure, relative to the pores of the gel. (See the discussion on native protein electrophoresis, page 10.) Large molecules can usually be expected to migrate more slowly than small ones, creating separation of the distinct particles within the gel.

By convention, polyacrylamide gels are characterized by %T, which is the weight percentage of total monomer including crosslinker (in g/100 ml). The %T gives an indication of the relative pore size of the gel. In general, pore size decreases with increasing %T. The %T can be calculated by the following equation.

$$\%T = \frac{\text{grams acrylamide} + \text{grams crosslinker}}{\text{total volume (ml)}} \times 100$$

The polyacrylamide %T can be made as a single continuous percentage throughout the gel, or it can be cast as a gradient of %T through the gel. Typical gel compositions are from 7.5% up to 20% for single percentage gels, or gradients ranging from 4-15% to 10-20%.

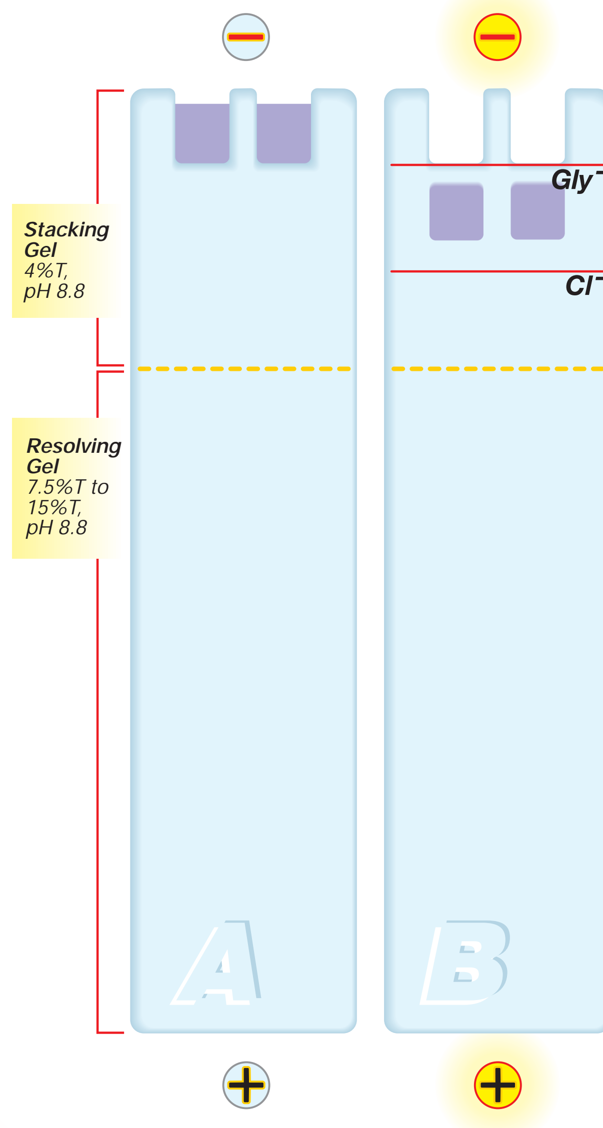
$$\%C = \frac{\text{grams crosslinker}}{\text{grams acrylamide} + \text{grams crosslinker}} \times 100$$

%C is the crosslinker ratio of the monomer solution. In general, pore size decreases with increasing %C.

Buffer Systems

The electrolyte buffer is a very important part of the electrophoresis system. It determines power requirements and affects separation. Sample proteins vary widely in their response to the constituents and make-up of the buffer. The buffer system is composed of both the buffer used in the gel and the running buffer. The two most common gel systems are ***continuous*** and ***discontinuous***.

> **Tip #1**
>
> ***Reusing buffers.*** *It's sometimes tempting to reuse running buffer. While there may be ionic species still available to carry current, be aware that there may be extraneous protein from the previous gel circulating in the running buffer. If used on another gel, it can cause higher background by depositing this protein into the new gel.*



Continuous Buffer Systems

Continuous buffer systems use the same buffer, at constant pH, in the gel and electrode reservoirs. The gel is typically made of one continuous gel %T and the sample is loaded directly into the part of the gel in which separation will occur. The advantage of this type of gel is in the ease of casting. Resolution of individual bands is usually not as good as on a discontinuous gel, since the bandwidth is determined somewhat by the height of the sample load. Continuous gel systems are most commonly used with gels for nucleic acid analysis.

Defining the Discontinuous Systems

Discontinuous buffer systems were devised initially for use with undenatured, or native, proteins. By using different buffers in the gel and in the electrode solutions, and adding a stacking gel to the resolving gel, the samples can be compressed into a thin starting band, from which finely resolved final bands of individual proteins are separated. A discontinuous gel has two main parts. The upper gel is known as a stacking gel and is made of a large pore matrix (typically 4%T) that acts as an anticonvective medium. The proteins pass easily through this matrix between leading and trailing ion fronts. Proteins become much more compressed into narrow starting zones by this method than is possible with any mechanical means. The lower resolving gel is made of a dense matrix that acts as a sieve. For a more detailed look at this process, see Figure 1.

Native (Non-Denaturing) Discontinuous Buffer System

The original discontinuous gel system, developed by Ornstein and Davis, was devised from electrochemical considerations based on the requirements of serum proteins. In this system, the electrophoretic migration is affected by the molecular weight, structure, and net charge of the protein. This can result in situations

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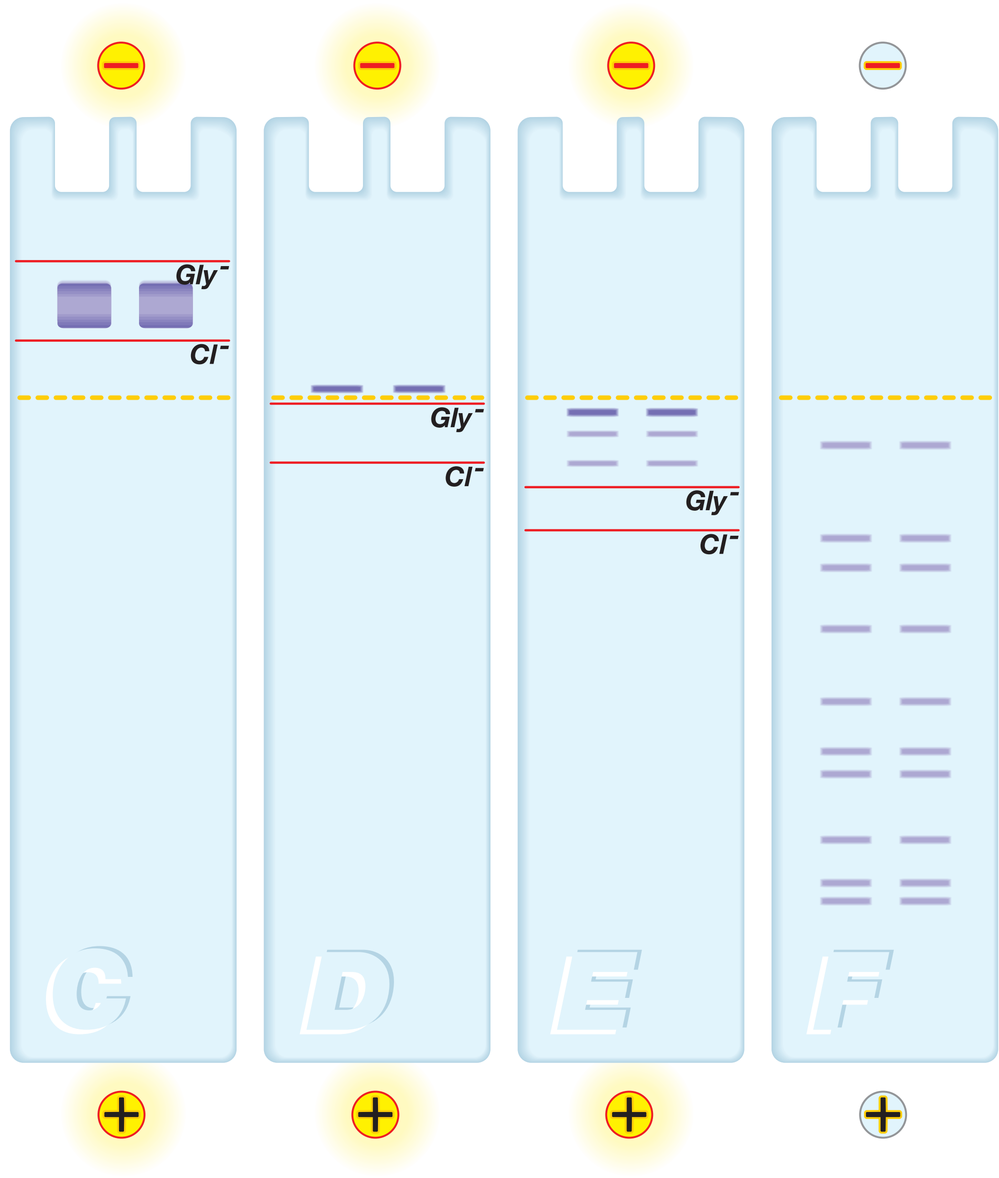

Fig. 1: Denaturing, discontinuous buffer system in Ready Gels. With Ready Gels, the stacking gel (4%T) is made at the same pH as the resolving gel. **A.** Denatured sample proteins are loaded into the wells. **B.** Voltage is applied and the samples move into the gel. The chloride ions already present in the gel run faster than the SDS-proteins and form an ion front. The glycinate ions flow in from the running buffer and form a front behind the proteins. **C.** A voltage gradient is created between chloride and glycinate ions, which sandwich the proteins in between. **D.** The proteins are tightly 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 molecular weight.

where high molecular weight proteins with high net negative charge migrate further into the gel than lower molecular weight, less strongly charged, proteins. For this reason, native gels are sometimes difficult to interpret. To overcome this net charge effect, Laemmli incorporated SDS into the system, producing a uniform charge-to-mass ratio on each protein, creating the denaturing discontinuous system.

Denaturing, Discontinuous Buffer System

By far the most popular gel system for separating proteins is SDS-PAGE, as devised by Laemmli (1970). In this system, proteins are denatured by heating them in buffer containing sodium dodecyl sulfate (SDS) and a thiol reducing agent such as 2-mercaptoethanol (ßME). The resultant polypeptides take on a uniform charge-to-mass ratio and rod-like dimensions imparted by the SDS, proportional to their molecular weights. The popularity of this system arises from its usefulness in estimating molecular weights.

Tip #2

Doublets or multiple bands when only one band is expected. *This is generally caused by insufficient reducing agent. Fresh 2-mercaptoethanol (ßME) should be spiked into sample buffer each time. ßME can volatilize off even when stored frozen, leading to insufficiently-reduced samples, so it should not be prepared in sample buffer ahead of time.*

Molecular Weight Estimation

Molecular weight estimations are among the most often used applications of gel electrophoresis and account in part for the popularity of the Laemmli SDS-PAGE method. Molecular weights are determined in SDS-PAGE by comparing the mobilities of test proteins to the mobilities of known protein markers. The relative mobility, R_f, is defined as the mobility of a protein divided by the mobility of the ion front. Because the ion front is difficult to locate in practice, mobilities can be normalized to the tracking dye that migrates only slightly behind the ion front: R_f = (distance to band)/(distance to dye front).

In each gel, a lane of standard proteins of known molecular weights is run in parallel with the test proteins. Plots of the logarithm of protein molecular weight (log Mr) versus the relative mobility, R_f, fit reasonably straight lines. The R_fs of the test proteins are then interpolated into the standard curve to give their approximate molecular weights.

Evolution to Ready Gels

Originally, SDS was added to the gels and buffers of the Laemmli system. It has been found, however, that SDS is not needed in the gel. The SDS in the sample buffer is sufficient to saturate the proteins, and the SDS in the cathode buffer is sufficient for maintaining saturation during electrophoresis. Because of this, precast Tris-HCl Ready Gels can be used for either native or SDS-PAGE applications, dependent only on the sample preparation and running buffer formulations.

Tris-Tricine Buffer System

One of the drawbacks of using SDS in a separation system is that the excess SDS runs as a large front at the low molecular weight end of the separation. Smaller polypeptides can be caught up in this front and prevented from resolving into discrete bands. Replacement of glycine with tricine results in 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 kDa can be separated in tricine gels. Stacking gels in this system are 4%T, and resolving gels of 16.5%T or 10-20%T gradient gels.

Choice of Systems

Native Proteins

There is no universal buffer system ideal for the electrophoresis of all native proteins. Protein stability, solubility, and the resolution required are important considerations in buffer selection. Discontinuous systems should always be considered for a new sample. However, in a discontinuous system, the concentration of proteins in the stacking gel can cause the proteins to aggregate and precipitate. In this case, they may not enter the resolving gel, or they may cause streaking as accumulated protein slowly dissolves during electrophoresis. Continuous systems may be appropriate in those situations where proteins aggregate upon stacking.

Percent Acrylamide

The choice of proper acrylamide concentration is also critical to the success of the separation. Too high %T can lead to exclusion of proteins from the gel, and too low %T can decrease sieving. Precast Ready Gels make this choice easy because of the many different percentage gels available. Precast gels eliminate the time-consuming process of casting different percentage gels to find the right gel percentage for the new sample.

Denatured Proteins

It is easier to choose suitable concentrations for SDS-PAGE gels than for native protein gels because separations are dependent mainly on polypeptide mass. Ready Gels are made in all the major gel percentages designed to fill the wide range of separation needs.

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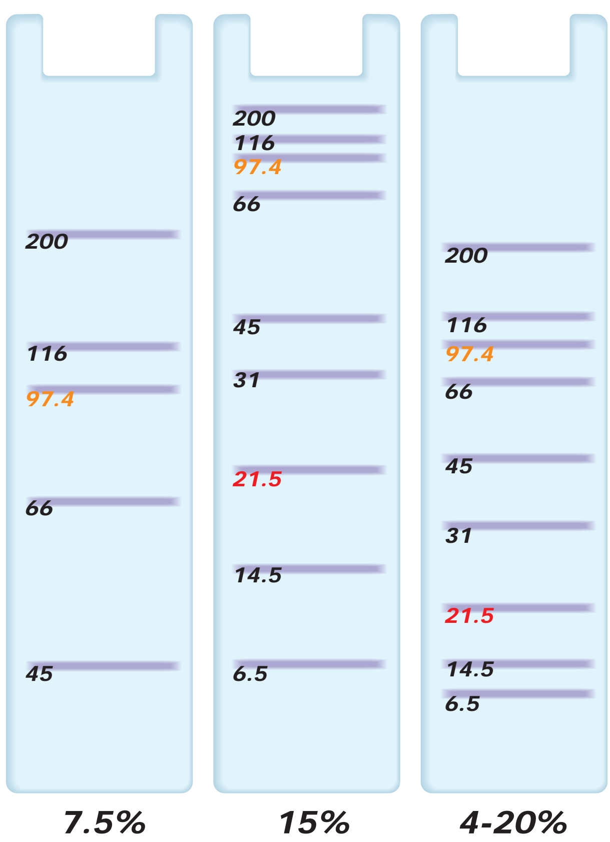

Fig. 2. Protein migration in single percentage vs. gradient SDS-PAGE.

Protein Migration in Single Percentage Versus Gradient Gels

The percentage of acrylamide determines the pore size within the gel matrix. Protein migration is affected by this concentration of acrylamide, and gels can be cast with a single %T throughout the resolving portion of the gel or as gradients of %T through the gel.

A gradient gel has an increasing concentration of acrylamide through the gel which results in decreasing pore sizes in the direction of migration. This allows separation of complex mixtures spanning wide molecular mass ranges. However, the best results in resolving two molecules from one another are achieved in single percentage gels. A common strategy for analysis of complex mixtures is to estimate the molecular weight and migration on a gradient gel and then perform more definitive analyses on an appropriate single percentage gel.

Figure 2 represents the range of resolution of 7.5% and 15% single percentage gels, and a 4-20% gradient gel. Note that in the single percentage gels, the lower and higher ranges (respectively) are not well resolved or are absent from the gel. The gradient gel resolves a wider range of molecular weights.

Special Purpose Gels

Zymogram Gel Analysis

Zymogram gels provide a means to detect and characterize collagenases and other proteases. The gel matrix incorporates casein or gelatin as a substrate for the protease. A positive result shows as an unstained band where proteases are present. The proteins are run with denaturing SDS in order to separate by molecular weight. A renaturation buffer is added to renature the enzymes to active forms, and then a development solution is added which provides a divalent metal cation required for enzymatic activity. A Coomassie R-250 stain is used to darkly stain the gel in order to create clear contrast for the clear protease bands.

Isoelectric Focusing

Isoelectric focusing (IEF) is an electrophoretic method for separating proteins in pH gradients. The net charge on a protein is determined by the pH of its local environment. When proteins move through a medium with varying pH, their net charges change in response to the pH they encounter. Under the influence of an electric field, a protein in a pH gradient will migrate until it focuses at the position in the gradient where its net charge is zero. Proteins show considerable variation in isoelectric points, but pI values usually fall in the range of pH 3 to pH 10.

Proteins are positively charged in solutions at pH values below their pI and negatively charged above their pI. Thus, at pH values below the pI of a particular protein, it will migrate toward the cathode during electrophoresis. At pH values above its pI, a protein will move toward the anode. A protein at its isoelectric point will not move in an electric field. This separation method will often reveal new and important insights into the sample protein, and can be easily processed with the advent of IEF Ready Gels. Figure 3 provides a visual reference of the IEF process.

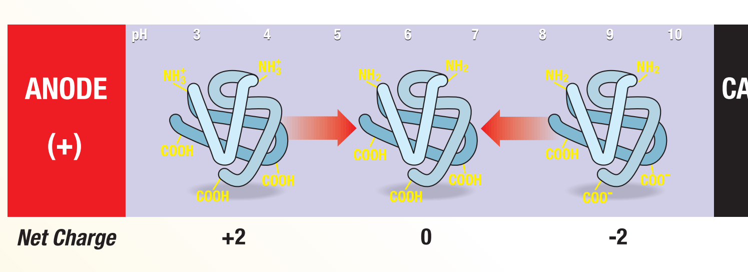

Fig. 3. Isoelectric focusing. A protein is depicted in a pH gradient in an electric field. A pH gradient formed by ampholyte molecules under the influence of an electric field is indicated. The gradient increases from acidic (pH 3) at the anode to basic (pH 10) at the cathode. The hypothetical protein 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 that 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 also becomes less negatively charged. When the protein becomes uncharged (pI), it ceases to move in the field and becomes focused.

Two Dimensional (2-D) Gel Electrophoresis

The resolving power of gel electrophoresis can be increased by combining two different techniques to produce a two-dimensional separation of the components in a sample. The most common two-dimensional technique is O'Farrell's method for proteins. Samples are first subjected to isoelectric focusing on a tube gel or gel strip, then to SDS-PAGE in a perpendicular direction which further separates the same proteins by molecular weight. Very high resolution two-dimensional methods have been developed, allowing thousands of polypeptides to be resolved in a single slab gel. The resulting “spots” can be visualized by gel staining, or transferred to a membrane support for total protein staining, or analysis with specific antibody detection. Ready Gels with the 2-D/Prep wells are ideal for 2-D screening of protein mixtures.

Sample Preparation

For native, discontinuous gels, the sample is typically diluted 2-fold to 5-fold in a dilution of the upper gel buffer. Tracking dye and glycerol (or sucrose) are added to the samples, for visibility and density, respectively.

Samples for SDS-PAGE by the Laemmli procedure are prepared in a Tris buffer containing SDS, 2-mercaptoethanol, glycerol, and bromophenol blue tracking dye. It is best to prepare a stock sample buffer containing everything except ß-mercaptoethanol, and to add this reagent right before use. The glycerol provides density for underlaying the sample on the stacking gel below the electrode buffer. The tracking dye allows visualization of the sample application, and migrates with the ion front so that the electrophoretic run can be monitored. The SDS added in the sample buffer is generally sufficient to ensure saturation of most protein mixtures. Premixed sample buffers, as well as premixed running buffers, are available to make the electrophoresis process fast and consistent.

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Electrophoresis Apparatus

Electrophoresis cells house the anode and cathode buffer compartments, electrodes (usually platinum wire), and jacks for making electrical contact with the electrodes. Acrylamide gels are held vertically between the electrode chambers while high voltage DC power supplies provide electrical power. The cells are responsible for much more than holding the gels and buffer, though. The results of electrophoresis are affected very much by the ability of the cells to dissipate heat, since uneven heat distribution on the gel is a main cause of band distortions.

The most popular gel systems used in research today are the mini-cells and mini-gel systems. Mini-gels allow rapid analysis and provide good resolution for a broad range of samples. The design of the mini-cell allows analyses to be completed much faster than is possible with larger cells. Run times as short as 35 minutes can be made with optimized gel and cell systems. Mini-gels can hold up to 15 samples and are very easy to handle. Due to their convenient size and durability, mini-gels have been made as precast gels in a wide variety of acrylamide percentage and comb types. See the extensive selection of precast Ready Gels on pages 26-34 of this catalog.

The Ready Gel Cell presents a new look in the way precast gels are run. It features a unique cam-lever sealing system, and its engineering includes a superior heat dissipation design that promotes exceptionally fast, uniform electrophoretic gel runs.

Tip #3

Dilution schemes. *When a protocol calls for a dilution, an often misunderstood nomenclature is used. The two parts are stated like a fraction, but not written that way. For example, "Dilute 1:2" means to take 1 part of one reagent and mix with 1 part of another, essentially diluting the 1 part by 1/2. 1:4 means to take 1 part and 3 parts, making a total of 4 parts, the 1 being diluted by 1/4. It is easier at higher dilutions; 1:20 is understood to mean 1 part plus 19 parts to make 20 total.*

Power Conditions

Regulated DC power supplies for electrophoresis should control voltage, current, and power conditions. All modes of operation can produce satisfactory results, but for best results and good reproducibility, some form of electrical control is important. The choice of which electrical parameter to control is almost a matter of preference.

The major limitation is the ability of the cell to dissipate the heat generated by the electrical current during an electrophoretic run. This Joule heat can have many deleterious consequences, such as band distortion, increased diffusion, enzyme inactivation, and protein denaturation. In general, electrophoresis should be performed at voltage and current settings at which the run proceeds as rapidly as the chamber's ability to draw off heat allows.

Electrical quantities are interrelated by fundamental laws. Each gel has an intrinsic resistance, (R), determined by the ionic strength of its buffer. When a voltage (V) is impressed across the gel, a current (I) flows through the gel and the external circuitry. These three quantities are related by Ohm's law: $V = IR$, where V is expressed in volts, I in amperes (amps), and R in ohms. In addition, power (P), in watts, is given by $P = IV$. Joule heating, (H), is related to power by the mechanical equivalent of heat, 4.18 joules/cal, or $H = P/4.18$, in cal/sec.

With the Ornstein-Davis and Laemmli systems, R increases during the course of electrophoresis. Thus, for runs at constant current in these gels, the voltage, power (I^2R), and, consequently, the heat of the gel chamber increase during the run. Under constant voltage conditions, current, power (V^2/R), and heat decrease during electrophoresis as R increases.

Constant current conditions, as a rule, result in shorter but hotter runs than do constant voltage runs. The increased run times of constant voltage conditions give increased time for the proteins to diffuse, but this appears to be offset by the temperature-dependent increase in diffusion rate of the constant current mode.

The Ready Gel Cell is extremely well engineered and dissipates heat very efficiently. With this cell, gels can be run at relatively high voltages resulting in very short run times for high quality resolution.

Detection and Visualization of Proteins in Gels

Proteins in gels can be detected by staining with dyes or metals. The sensitivity and staining action varies between the stains. The following is a brief summary of each stain. See page 44 in the Staining Section of this catalog for a more direct comparison of sensitivities and use.

Coomassie Brilliant Blue R-250 is the most common protein stain. This stain penetrates into the gel and binds to the protein within the gel. A different Coomassie, Brilliant Blue G-250, should be used for staining gels containing low molecular weight polypeptides. The G-250 is a colloidal suspension of dye and binds to the proteins closest to the surface first. Over a longer staining period, it does slowly penetrate the gel and bind to more of the protein.

Silver staining is the most sensitive method for staining proteins and nucleic acids in gels. Bio-Rad offers two silver stains, the classic Merrill stain and Silver Stain Plus, for rapid, high sensitivity, low background staining.

Zinc and Copper stains are rapid, negative stains for SDS-PAGE. These stains do not fix the protein in the gel and do not add any dye to the protein directly. The protein bands show up as clear areas in a white or blue background, respectively. They are easily destained and allow use of the protein after visualization for further analytical techniques.

Recently, fluorescent stains have been introduced, such as SYPRO Orange fluorescent protein stain. This is a general stain which binds to all SDS coated proteins. This stain allows for documentation with a gel imaging instrument, such as Gel Doc 1000 or Fluor-S MultiImager system.

Protein Detection in Western Blotting

When proteins are transferred from a gel onto a membrane, they are readily accessible to antibody probes. This has led to the development of a variety of highly specific and sensitive assays collectively known as blots. Probing of membrane-bound proteins is generally done immunologically with antibodies, and is known as immuno-blotting. For historical reasons, it is also called western blotting.

A typical immunoblotting experiment consists of six interrelated steps. (1) Proteins are first fractionated by electrophoresis in a polyacrylamide gel. (2) The proteins are then transferred from the gel to a membrane where they become immobilized as a replica of the gel's band pattern. (3) Next, unoccupied protein-binding sites on the membrane are saturated to prevent non-specific binding of antibodies. (4) The blot is then probed for the proteins of interest with specific, primary antibodies. (5) Secondary antibodies, specific for the primary antibody type and conjugated to detectable reporter groups, such as enzymes or radioactive isotopes, are then used to label the primary antibodies. (6) Finally, the labeled protein bands are made visible by the bound reporter groups acting on an added substrate, or by radioactive decay.

Tip #4

How do I get the best sensitivity with SYPRO Orange? *Run a gel with narrow lanes (15-well comb), and use a photo or gel documentation system to visualize the gel. A gel on a transilluminator read by eye is not as sensitive to low level fluorescence as an instrument.*

Tip #5

Reducing background on chemiluminescent blots. *The blocking agent concentration and antibody dilutions are the two most important factors in blotting. Good blockers are non-fat dry milk, casein, and BSA. The working range is normally from 0.2% - 5% (more is not always better). Antibody titers should be done to optimize primary and secondary antibody dilutions. Use dot blot serial dilutions of antigen or antibody to quickly work out an optimum protocol.*

Summary

Electrophoresis is a powerful resolving technique applicable to a wide range of biological molecules. This section has provided an overview of the basic concepts involved in electrophoresis, and is intended to highlight areas important to the process of obtaining the desired results.

The following sections of this catalog expand on the power of electrophoresis through products designed to take you through this process faster, more efficiently, and with better results than ever before.

References:
Ornstein, L. (1964). Ann. *N.Y. Acad. Sci.* **121,** 321-349.
Laemmli, U.K. (1970). *Nature* (London) **227,** 680-685.

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Ready Gel System Applications
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Applications:

- *Protein Molecular Weight Analysis*
- *Native Protein Analysis*
- *Peptide Analysis*
- *Isoelectric Point Analysis/IEF*
- *Protease Analysis*
- *Glycoprotein Analysis*
- *Oligosaccharide Analysis*
- *dsDNA Analysis*
- *ssDNA, RNA Analysis*

Applications for Ready Gels

The wide variety of Ready Gels makes common applications easier, and unusual applications more accessible. Use the Applications & Systems Guide for each application on the next several pages to determine which reagents complement the particular technique you intend to run.

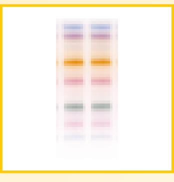
The Ready Gel System:

Precast Ready Gels

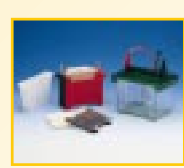
Ready Gel Cell

Power Supplies

Premixed Buffers



Standards



Detection Systems

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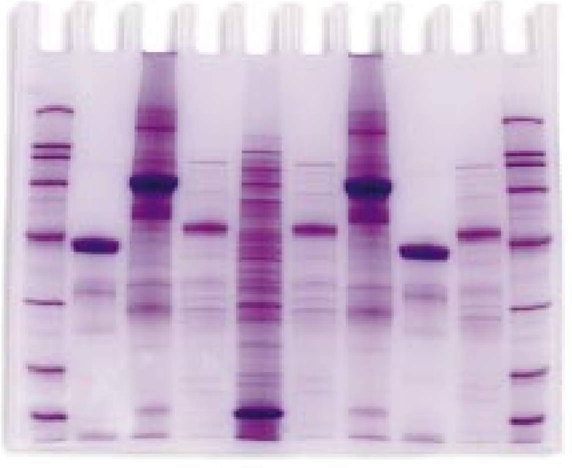
Step	Ready Gel System Product		Cat. No.	Suggested Standard	Cat. No.
Sample Preparation	Laemmli Sample Buffer		161-0737		
	2-mercaptoethanol		161-0710		
Ready Gel and Standard Selection	**MW Range Resolved**	**Tris-HCl Gel %T**	**10 Well, 10 Pack**	**SDS-PAGE**	
	100-250 kDa	5%	161-1052	High range	161-0303
	40-200 kDa	7.5%	161-0900	Broad range	161-0317
	30-150 kDa	10%	161-0907	Broad range	161-0317
	20-120 kDa	12%	161-0901	Broad range	161-0317
	10-100 kDa	15%	161-0908	Low range	161-0304
	6-50 kDa	18%	161-1058	Low range	161-0304
	20-250 kDa	4-15%	161-0902	Broad range	161-0317
	10-200 kDa	4-20%	161-0903	Broad range	161-0317
	6-70 kDa	8-16%	161-1064	Broad range	161-0317
	10-100 kDa	10-20%	161-0906	Broad range	161-0317
Running Buffer	Tris-Glycine/SDS		161-0732		
Electrophoresis Cell and Power Supply	Ready Gel Cell		165-3125		
	PowerPac 300 (100/120 V)		165-5050		
	PowerPac 300 (220/240 V)		165-5051		
	Run conditions per 2 gels: Begin - 200 V (C), 100 mA, 35 minutes; End - 200 V (C), 60 mA				
Protein Stain	Coomassie R-250 Kit		161-0435		
	Silver Stain Plus Kit		161-0449		
	Bio-Rad Silver Stain Kit		161-0443		
	SYPRO Orange Fluorescent Stain		170-3120		
	~~Zinc Stain Kit~~		~~161-0440~~		
Alternative Detection: Western Blotting	Transfer Equipment, Membranes, and Detection Reagents				

For more information on:
Ready Gels - Single packs, 10 packs, and 20 packs, see page 29
Buffer formulation - see page 42
Standards - Prestained, Silver, and SYPRO Orange, see pages 50-51
Stains sensitivity - see page 44
Western blotting - see page 55

The most common application for protein electrophoresis is the separation of complex protein mixtures by molecular weight. This is a basic characterization tool, as well as a high resolution method for assessing protein purity. Proteins are denatured in solutions containing SDS. The detergent coats the proteins and imparts a uniform negative charge to them. This overwhelms any natural charges proteins have, and allows proteins to be compared by molecular weight.

Tip #6

Running denatured or native. *The difference between running a Ready Gel as SDS-PAGE or native depends on the sample preparation and the running buffer. The same Tris-HCl Ready Gel or Tris-Tricine Ready Gel is run for either system. There is enough SDS and denaturant in the sample buffer and SDS in the running buffer to completely denature the protein during electrophoresis.*



12% Tris-HCl Ready Gel, 10 well, catalog number 161-1102. Run with molecular weight standards and samples, then stained with Coomassie R-250.

Step	Ready Gel System Product	Cat. No.
Sample Preparation	Native Sample Buffer	161-0738
Ready Gel Selection	**Tris-HCl Gel %T**	**10 Well, 10 Pack**
	5%	161-1052
	7.5%	161-0900
	10%	161-0907
	12%	161-0901
	15%	161-0908
	18%	161-1058
	4-15%	161-0902
	4-20%	161-0903
	8-16%	161-1064
	10-20%	161-0906
Running Buffer	Tris-Glycine	161-0734
Electrophoresis Cell and Power Supply	Ready Gel Cell	165-3125
	PowerPac 300 (100/120 V)	165-5050
	PowerPac 300 (220/240 V)	165-5051
	Run conditions per 2 gels: Begin - 200 V (C), 100 mA, 35 minutes End - 200 V (C), 60 mA	
Protein Stain	Coomassie R-250 Kit	161-0435
	Silver Stain Plus Kit	161-0449
	Bio-Rad Silver Stain Kit	161-0443
Alternative Detection: Western Blotting	Transfer Equipment, Membranes, and Detection Reagents	
	Transfer Buffer: Tris-Glycine	161-0734

For more information on:
Ready Gels - Single packs, 10 packs, and 20 packs, see page 29
Buffer formulations - see page 42
Stains sensitivity - see page 44
Western blotting - see page 55

The Ready Gel System provides a platform for applications that run proteins in their native form. Non-denaturing conditions are used with Tris-HCl Ready Gels to separate complex mixtures of non-denatured proteins for analysis of native conformation and activity. This will not provide molecular weight information because the native form will migrate based on net charge, globular shape, and mass.

Tip #7

Streaking in the lane. *If the sample looks streaky or smeary, it is probably due to the sample proteins coming out of solution due to partial insolubility, precipitation, or aggregation. Optimization of the sample buffer and running conditions needs to be considered.*

10% Tris-HCl Ready Gel, protein samples run non-denatured, then stained with Coomassie R-250.

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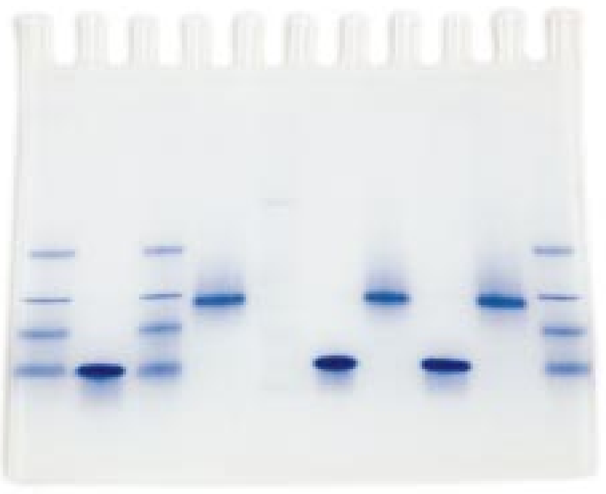
Step	Ready Gel System Product		Cat. No.	Suggested Standard	Cat. No.
Sample Preparation	Tricine Sample Buffer		161-0739		
	2-mercaptoethanol		161-0710		
Ready Gel and Standard Selection	MW Range Resolved	Tris-Tricine Gel %T	10 Well, 10 Pack	SDS-PAGE	
	4-30 kDa	16.5%	161-0922	Polypeptide	161-0326
	5-40 kDa	10-20%	161-0923	Polypeptide	161-0326
Running Buffer	Tris-Tricine/SDS		161-0744		
Electrophoresis Cell and Power Supply	Ready Gel Cell		165-3125		
	PowerPac 300 (100/120 V)		165-5050		
	PowerPac 300 (220/240 V)		165-5051		
	Run conditions per 2 gels: Begin - 100 V (C), 65 mA, 100 minutes End - 100 V (C), 35 mA				
Protein Stain	Coomassie G-250		161-0406		
	Silver Stain Plus Kit		161-0449		
	Bio-Rad Silver Stain Kit		161-0443		
	SYPRO Orange Fluorescent Stain		170-3120		
	Zinc Stain Kit		161-0440		
Alternative Detection: Western Blotting	Transfer Equipment, Membranes, and Detection Reagents				
	Transfer Buffer: Tris-Glycine		161-0734		

For more information on:
Ready Gels - Single packs, 10 packs, and 20 packs, see page 30
Buffer formulations - see page 42
Standards - Prestained, Silver, and SYPRO Orange, see pages 50-51
Stains sensitivity - see page 44
Western blotting - see page 55

The analysis of small proteins and polypeptides requires a buffer system and acrylamide percentages especially suited to the small molecules. One weakness of the Tris-Glycine buffer system is that small protein and polypeptide molecules will run with the SDS front. The Tricine buffer system separates small SDS coated proteins from SDS micelles due to the mobility of tricine. Tricine SDS-PAGE has also proven useful for resolving mixtures of lipopolysaccharides and lipooligosaccharides.

Tip #8

Running buffers. *When preparing running buffers for any experiment, make the solution to the molarity – do not titrate to a pH. The ion balance has been set by the concentration of reagents; adjusting the pH will alter this balance and lead to undesirable results.*



10-20% Tris-Tricine Ready Gel, 10 well, catalog number 161-0923. The gel was run with molecular weight standards and samples, then stained with Coomassie G-250.

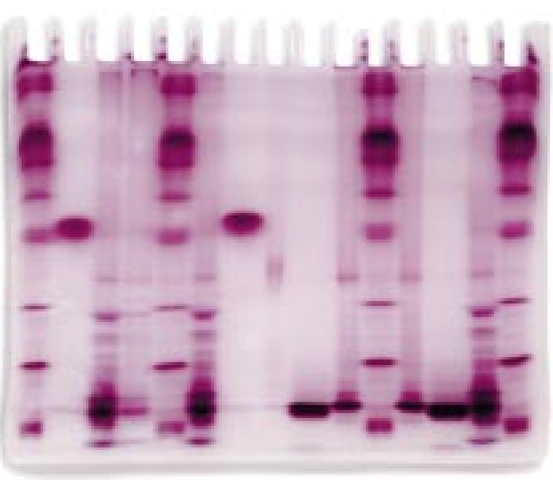
Step	Ready Gel System Product	Cat. No.	Suggested Standard	Cat. No.
Sample Preparation	IEF Sample Buffer	161-0763		
Ready Gel and Standard Selection	pH Range Resolved / IEF Gel %T	10 Well, 10 Pack		
	3-10 / 5%	161-1010	IEF Standard	161-0310
	5-8 / 5%	161-1016	IEF Standard	161-0310
Running Buffer	Anode Buffer	161-0761		
	Cathode Buffer	161-0762		
Electrophoresis Cell and Power Supply	Ready Gel Cell	165-3125		
	PowerPac 1000 (100/120 V)	165-5054		
	PowerPac 1000 (220/240 V)	165-5055		
	Run conditions per 2 gels stepwise: 100 V (C), 5-25 mA, 1 hour 250 V (C), 5-25 mA, 1 hour 500 V (C), 5-25 mA, 30 minutes			
Gel Stain	Premixed IEF Stain	161-0434		
	Silver Stain Plus Kit	161-0449		
	Bio-Rad Silver Stain Kit	161-0443		
Alternative Detection: Western Blotting	Transfer Equipment, Membranes, and Detection Reagents			

For more information on:
Ready Gels - Single packs, 10 packs, and 20 packs, see page 31
Buffer formulations - see page 42
Standards - IEF, see page 52
Stains sensitivity - see page 44
Western blotting - see page 55

Non-denaturing isoelectric focusing (IEF) separates proteins by net charge rather than molecular weight. Gels cast with amphoteric molecules set up pH gradients throughout the gels, and the proteins migrate to their neutral pI point. Unlike the common first dimension IEF gels and strips used in 2-D electrophoresis, IEF slab gels are run vertically with a continuous, non-denaturing pI gradient through the gel.

Tip #9

Running conditions for IEF Ready Gels. *IEF gels require high voltage with step changes at specified time points. It is best to use a programmable power supply capable of handling both the high voltage and a preprogrammed sequence of timed voltage changes. The Bio-Rad PowerPac 1000 and 3000 power supplies are ideal for this technique.*



3-10 range IEF Ready Gel, 15 well, catalog number 161-1129. The gel was run with IEF standards and samples, then stained with IEF Stain (Coomassie R-250 / Crocein Scarlet).

Step	Ready Gel System Product		Cat. No.	Suggested Standard	Cat. No.
Sample Preparation	Zymogram Sample Buffer		161-0764		
Ready Gel and Standard Selection	MW Range Resolved	Zymogram Gel %T With Contents	10 Well, 10 Pack	Prestained	
	30-150 kDa	10% with Gelatin	161-1022	Broad range	161-0318
	20-120 kDa	12% with ß-Casein	161-1028	Broad range	161-0318
Running Buffer	Tris-Glycine/SDS		161-0732		
Electrophoresis Cell and Power Supply	Ready Gel Cell		165-3125		
	PowerPac 300 (100/120 V)		165-5050		
	PowerPac 300 (220/240 V)		165-5051		
	Run conditions per 2 gels: Begin - 100 V (C), 25 mA, 80-100 minutes End - 100 V (C), 12 mA				
Renaturation	Zymogram Renaturation Buffer		161-0765		
Development	Zymogram Development Buffer		161-0766		
Gel Staining	Coomassie R-250, 0.5% solution		161-0400		

For more information on:
Ready Gels - Single packs, 10 packs, and 20 packs, see page 32
Buffer formulations - see page 42
Standards - Prestained, see page 50
Stains sensitivity - see page 44

It is often useful to test for proteolytic activity when performing protein characterizations. Among the possible methods, Zymogram Gels are a popular approach. Gels are cast with gelatin or casein which act as substrates for proteases. A positive result, following renaturation and Coomassie staining, is distinguished by a clear band in a darkly stained gel.

Tip #10

Zymogram staining. *Use a 0.5% Coomassie R-250 stain solution, instead of the regular 0.1% solution, because the Zymogram gel absorbs much more stain than a regular gel. The darker stain helps you visualize the contrast of a positive result (a clear band).*

10% Zymogram Ready Gel with Gelatin, 10 well, catalog number 161-1022.

Step	Ready Gel System Product		Cat. No.	Suggested Standard	Cat. No.
Sample Preparation	Laemmli Sample Buffer		161-0737		
	2-mercaptoethanol		161-0710		
Ready Gel and Standard Selection	**MW Range Resolved**	**Tris-HCl Gel %T**	**10 Well, 10 Pack**	**SDS-PAGE**	
	100-250 kDa	5%	161-1052	High range	161-0303
	40-200 kDa	7.5%	161-0900	Broad range	161-0317
	30-150 kDa	10%	161-0907	Broad range	161-0317
	20-120 kDa	12%	161-0901	Broad range	161-0317
	10-100 kDa	15%	161-0908	Low range	161-0304
	6-50 kDa	18%	161-1058	Low range	161-0304
	20-250 kDa	4-15%	161-0902	Broad range	161-0317
	10-200 kDa	4-20%	161-0903	Broad range	161-0317
	6-70 kDa	8-16%	161-1064	Broad range	161-0317
	10-100 kDa	10-20%	161-0906	Broad range	161-0317
Running Buffer	Tris-Glycine/SDS		161-0732		
Electrophoresis Cell and Power Supply	Ready Gel Cell		165-3125		
	PowerPac 300 (100/120 V)		165-5050		
	PowerPac 300 (220/240 V)		165-5051		
	Run conditions per 2 gels: Begin - 200 V (C), 100 mA, 35 minutes; End - 200 V (C), 60 mA				
Detection Method	Glycoprotein Detection Colorimetric Western Blot (BCIP/NBT)		170-6490		
	Deglycosylation Detection Coomassie R-250 Kit		170-6500		

For more information on:
Ready Gels - Single packs, 10 packs, and 20 packs, see page 29
Buffer formulations - see page 42
Standards - Prestained and SDS-PAGE, see pages 50-51
Stains sensitivity - see page 44
Western blotting - see page 55
Glycoprotein kits and reagents - see page 48

Complex carbohydrates are important components of all living things. In addition to providing energy and structural support for cells, increasing evidence has shown that the carbohydrate moieties of glycoproteins are often important as recognition determinants in receptor-ligand or cell-cell interactions, in the modulation of immunogenecity and protein folding, and in the regulation of protein bioactivity.

Tip #11

Is my protein glycosylated? *Using a western blot procedure, this question can be answered using the Immun-Blot Kit for Glycoprotein Detection.*

Coomassie stained 12% Ready Gel. Human transferrin and ovalbumin were deglycosylated with the Enzymatic Deglycosylation Kit. Mobility shift indicates proteins were deglycosylated.

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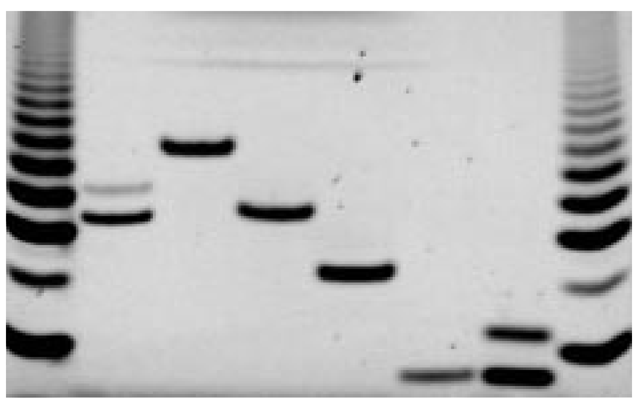
Step	Ready Gel System Product / Cat. No.		
Sample Preparation Kit Selection	**N-Link Oligosaccharide Analysis** Glycoprotein in non-Tris buffer buffer Profiling Kit / 170-6501 Sequencing Kit / 170-6510	**O-Link Oligosaccharide Analysis** Glycoprotein in non-Tris buffer Profiling Kit / 170-6815	**Monosaccharide Analysis** Glycoprotein in non-Tris Composition Kit / 170-6811
Running Buffer	Oligosaccharide Buffer Pack 170-6503	Oligosaccharide Buffer Pack 170-6503	Monosaccharide Buffer Pack 170-6814
Electrophoresis Cell and Power Supply	Ready Gel Cell / 165-3125 Carbohydrate Gaskets 165-3157 PowerPac 300 (100/120 V) 165-5050 PowerPac 300 (220/240 V) 165-5051 Run conditions per 2 gels: Begin: 100-200 V, 30 mA (C), 60-90 minutes End: 200-300 V, 30 mA (C)	Ready Gel Cell / 165-3125 Carbohydrate Gaskets 165-3157 PowerPac 300 (100/120 V) 165-5050 PowerPac 300 (220/240 V) 165-5051 Run conditions per 2 gels: Begin: 200-300 V, 40 mA (C), 50-60 minutes End: 600-700 V, 40 mA (C)	Ready Gel Cell / 165-3125 Carbohydrate Gaskets 165-3157 PowerPac 300 (100/120 V) 165-5050 PowerPac 300 (220/240 V) 165-5051 Run conditions per 2 gels: Begin: 300-400 V, 60 mA (C), 50-60 minutes End: 500-600 V, 60 mA (C)
Detection Method	ANTS Fluorophore	ANTS Fluorophore	AMAC Fluorophore
Imaging Options	Gel Doc 1000 or	Gel Doc 1000 or	Gel Doc 1000 or

The potential for oligosaccharide diversity is great, but the biosynthesis of N-linked oligosaccharides consists of a structured set of consecutive reactions. Consequently, this potential diversity is minimized and some simple prediction rules for N-linked oligosaccharide structural analysis can be utilized. Conversely, O-linked oligosaccharides are heterogeneous with a variety of core structures, making O-linked oligosaccharide structural analysis more complex.

Tip #12

What is the monosaccharide sequence of the oligosaccharide? *The N-Linked Oligosaccharide Sequencing Kit uses exoglycosidases and polyacrylamide gel electrophoresis to determine the sequence of N-linked oligosaccharides.*



N-Linked Oligosaccharide Sequencing. A sialylated, galatosylated, bi-antennary oligosaccharide sequenced with the N-Linked Oligosaccharide Sequencing Kit.

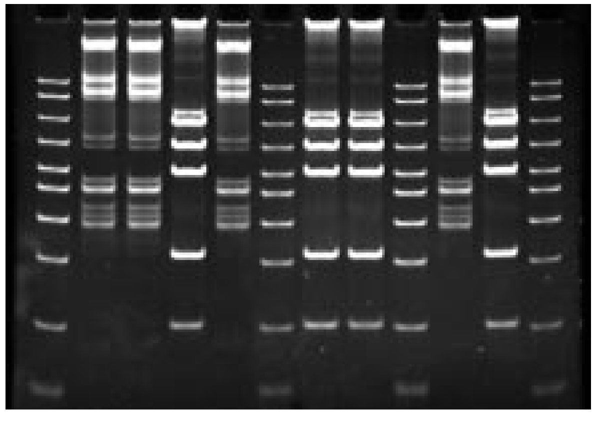
Step	Ready Gel System Product		Cat. No.	Suggested Standard	Cat. No.
Sample Preparation	TBE Sample Buffer		161-0767		
	MW Range Resolved	TBE Gel %T	10 Well, 10 Pack		
Precast Gel Selection	200-2,000 bp	5%	161-0904	AmpliSize	170-8200
	50-1,500 bp	10%	161-0905	AmpliSize	170-8200
	20-1,000 bp	15%	161-1070	20 bp	170-8201
	10-2,000 bp	4-20%	161-1077	AmpliSize	170-8200
Running Buffer	TBE		161-0733		
Electrophoresis Cell and Power Supply	Ready Gel Cell		165-3125		
	PowerPac 300 (100/120 V)		165-5050		
	PowerPac 300 (220/240 V)		165-5051		
	Run conditions per 2 gels: Begin - 100 V (C), 26 mA, 45-105 minutes End - 100 V (C), 22 mA				
Gel Staining	Ethidium Bromide Solution		161-0433		
	Silver Stain Plus Kit		161-0449		
	Bio-Rad Silver Stain		Kit		

For more information on:
Ready Gels - Single packs, 10 packs, and 20 packs, see page 33
Buffer formulations - see page 42
Standards - Nucleic acid, see page 53
Stains sensitivity - see page 44

Polyacrylamide TBE gels are an excellent means for high resolution separations of DNA fragments up to 2,000 bp. This is ideal for analyzing the purity of PCR fragments, and many other non-denatured DNA and RNA applications. TBE Ready Gels provide a consistent format for resolving these DNA fragments.

Tip #13

Sample load volumes in TBE gels. *Make the sample volumes as small as possible to keep the bands running as tight as possible. The gel will condense the sample to some extent, but it helps to start with a small volume of sample.*



5% TBE Ready Gel, 12 well, catalog number 161-1181. Ethidium Bromide stain.

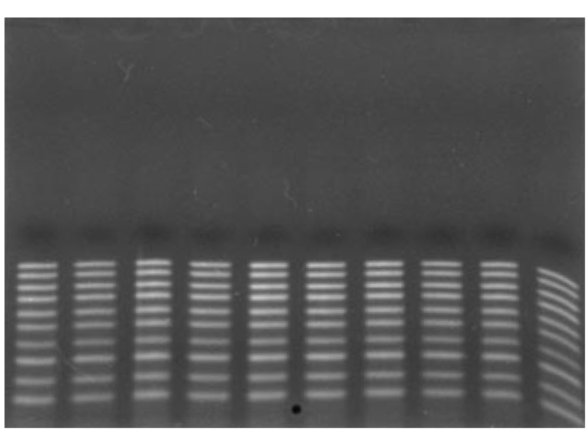
Step	Ready Gel System Product		Cat. No.
Sample Preparation	TBE-Ficoll Sample Buffer		
Precast Gel Selection	**MW Range Resolved**	**TBE-Urea Gel %T**	**10 Well, 10 Pack**
	50-1,000 bases	5%	161-1034
	25-200 bases	10%	161-1040
	10-50 bases	15%	161-1046
Running Buffer	TBE		161-0733
Electrophoresis Cell and Power Supply	Ready Gel Cell		165-3125
	PowerPac 300 (100/120 V)		165-5050
	PowerPac 300 (220/240 V)		165-5051
	Run conditions per 2 gels: Begin - 200 V (C), 30 mA, 40-90 minutes End - 200 V (C), 20 mA		
Gel Staining	Ethidium Bromide Solution		161-0433
	Radiant Red RNA Stain Solution		170-3122

For more information on:
Ready Gels - Single packs, 10 packs, and 20 packs, see page 34
Buffer formulations - see page 42
Stains - see page 44

TBE-Urea Ready Gels are polyacrylamide gels containing 7 M Urea. This produces a gel that maintains denaturing conditions for analysis of single stranded DNA and RNA. Applications include oligonucleotide purity analysis, RNAse protection assays, and northern blotting. TBE-Urea gels are an excellent matrix for high resolution separations of ssDNA and RNA fragments from 10 to 1,000 bases.

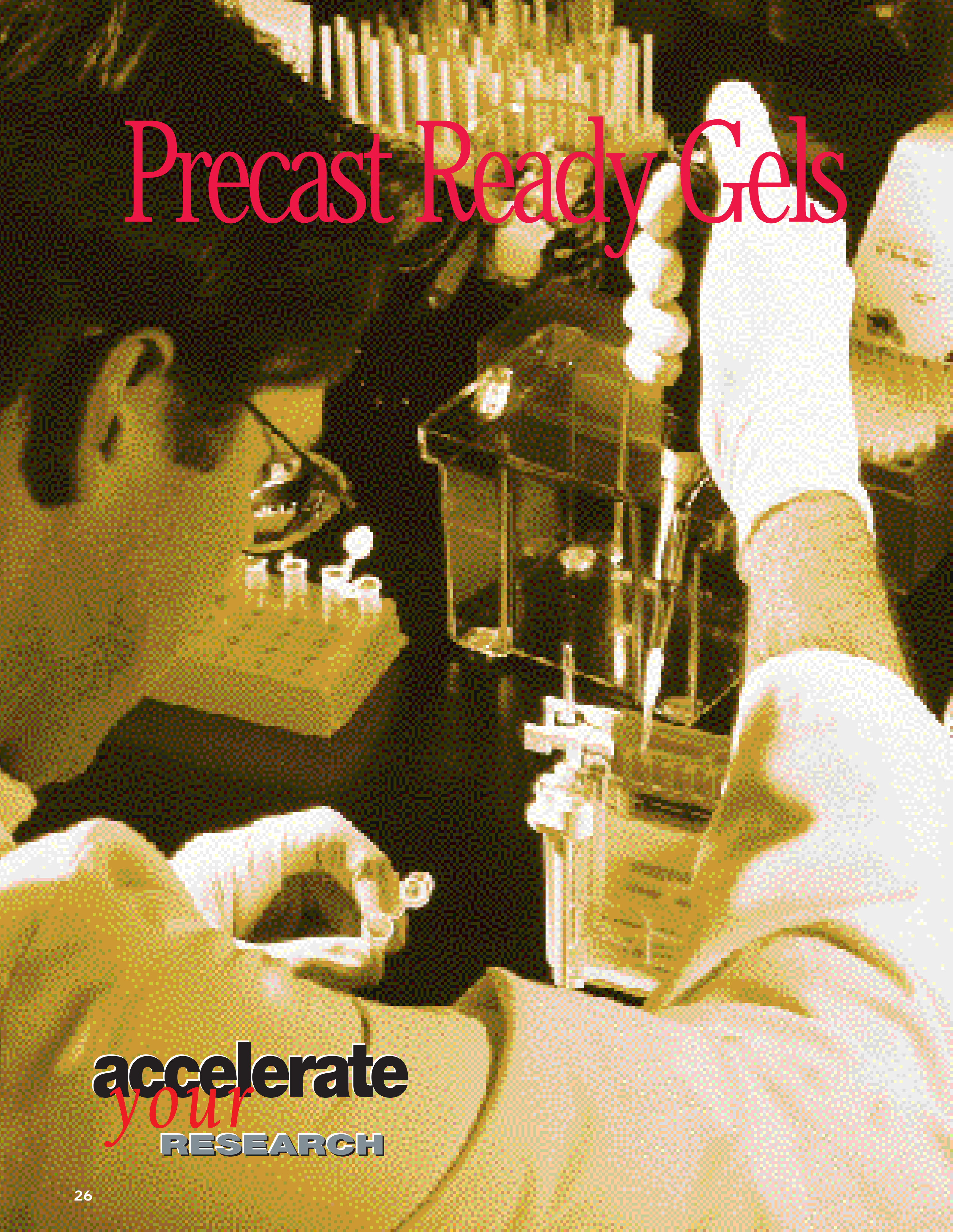
Tip #14

Wash out the urea. *After setting the TBE-Urea gels in the tank, rinse out the wells with running buffer before loading samples. The urea cast in the gel will start to come out of the gel and can disrupt sample loading. Once the power is on, the samples will have no problem running into the gel.*



15% TBE-Urea Ready Gel, 10 well, catalog number 161-1117. Ethidium Bromide stain.

Precast Ready Gels



Like conventional precast polyacrylamide gels, Ready Gels are designed to be used in a mini vertical electrophoresis system – in this case, the Ready Gel Cell. But Ready Gels are much easier to use. Setup in the Ready Gel Cell takes just seconds. They can be run in as little as 35 minutes, and opening them is effortless. Ready Gels provide consistent results with sharply resolved bands, while saving valuable preparation and running time. With Ready Gels, you can complete your entire electrophoresis run in less time than it takes just to prepare a gel from scratch.

- ***Fast results.*** Setup in the Ready Gel Cell takes just seconds, and runs are completed in as little as 35 minutes.
- ***Reproducible quality.*** Produced with high quality electrophoresis reagents with stringent quality control and functional testing for guaranteed results.

Selection

Ready Gels come in a wide variety of acrylamide percentages, from single percentage gels with stackers to linear gradients. In addition, Bio-Rad offers gel types and comb configurations to meet virtually every electrophoresis application.

Ready Gel Selection Guide

Gel Type	Page No.	Gel Formats	Packaging	Available Comb Types
Tris-HCl	29	Single %T or Gradient %T 2.6 %C	Single Gels 10 Packs 20 Packs	All
Tris-Tricine	30	Single %T or Gradient %T 3.3 %C	Single Gels 10 Packs 20 Packs	All
IEF	31	Single %T 3 %C	Single Gels 10 Packs 20 Packs	All
Zymogram	32	Single %T 2.6 %C	Single Gels 10 Packs 20 Packs	All except 2-D/Prep
TBE	33	Single %T or Gradient %T 3.3 %C	Single Gels 10 Packs 20 Packs	All except 2-D/Prep
TBE-Urea	34	Single %T 3.3 %C	Single Gels 10 Packs 20 Packs	All except 10 well/50 µl and 15 well

Ready Gel Combs

Comb Types	Volume
9 well (Octapette compatible)	30 µl
10 well	30 µl
10 well/50 µl	50 µl
12 well	20 µl
15 well	15 µl
2-D/Prep	450 µl

See page 37 for Ready Gel Comb ordering information.

Material Specifications for Ready Gels

Gel Material	Polyacrylamide
Gel Dimensions	Height: 7.0 cm, Width: 8.3 cm, Thickness: 1.0 mm
Cassette Materials	Back (long) plate: Acrylic, Front (short) plate: Glass
Cassette Dimensions	Height: 8.3 cm, Width: 10.0 cm
Comb Material	Polycarbonate
Storage	4 °C, do not freeze
Shelf Life	Most: 4 months; IEF: 6 months
Storage Buffer	Aqueous buffer with 0.02% NaN_3
Instruction Manual	Available upon request when purchasing Ready Gels. Cat. No. 161-0993
Key Knife	Available upon request when purchasing Ready Gels. Cat. No. 161-0992

Tris-HCl Ready Gels are formulated for use with SDS-PAGE and native PAGE systems. An excellent general purpose gel, the basic pH of the Tris-HCl system assures uniform charges on proteins, and the wide variety of gel percentages allows a broad range of proteins to be separated. Tris-Glycine buffer systems utilize Tris-HCl gels, in particular the Laemmli buffer system, which is easily the most commonly used and referenced PAGE system available.

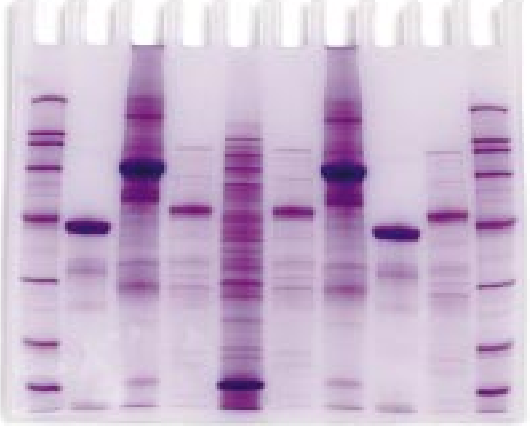
- ***Excellent general purpose gels***
- ***Wide variety of gel percentages allows a broad range of separations***

Tip #15

SDS - the Laemmli System. *Tris-HCl Ready Gels can be used for standard SDS-PAGE systems as well as non-denaturing native systems because they are cast without SDS. In reducing-denaturing systems (Laemmli), the sample buffer and running buffer contain sufficient SDS to keep proteins denatured during electrophoresis. When non-denaturing native-PAGE conditions are needed, the same gel can be used with the appropriate buffers.*

Single Percentage Ready Gels

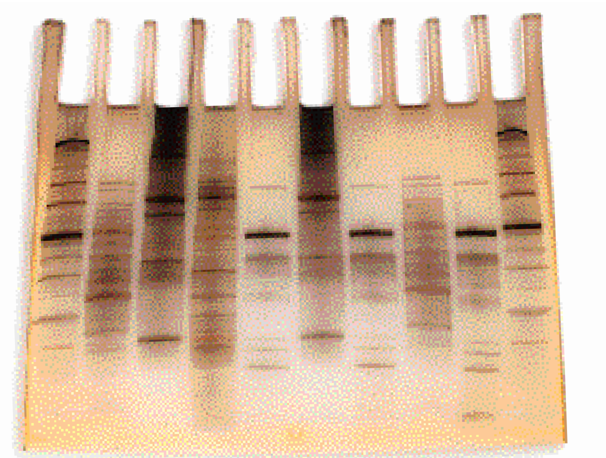
Single percentage gels allow maximum separation of protein bands of interest, and provide maximum blotting efficiency. Each gel is cast with a uniform concentration resolving gel and a 4% stacking gel. New gel percentages are introduced on a regular basis, so check with your local Bio-Rad representative if you do not see the gel percentage you need.



12% Tris-HCl Ready Gel, 10 well, catalog number 161-1102. Samples: lanes 1, 10 Broad Range SDS-PAGE Standards; lanes 2, 8 Alcohol Dehydrogenase; lanes 3, 7 Skunk Serum lysate; lanes 4, 6, 9 Urease; lane 5 *E. Coli* lysate. Stained with Coomassie R-250.

Gradient Percentage Ready Gels

Linear gradient gels provide superior resolution of samples containing a wide range of molecular weights. The automated casting systems used to produce Ready Gels create a completely linear gradient with a reproducibility far surpassing that of hand casting. Tris-HCl Ready Gels are available in four gradients, but custom gradients can be made upon request.



10-20% gradient Tris-HCl Ready Gel, 50 µl well, catalog number 161-1160. Samples: lanes 1, 10 Broad Range SDS-PAGE Standards; lanes 2, 8 Urease; lanes 3, 6 Skunk Serum lysate; lane 4 *E. Coli* lysate; lanes 5, 7, 9 Alcohol Dehydrogenase. Stained with Silver Stain Plus.

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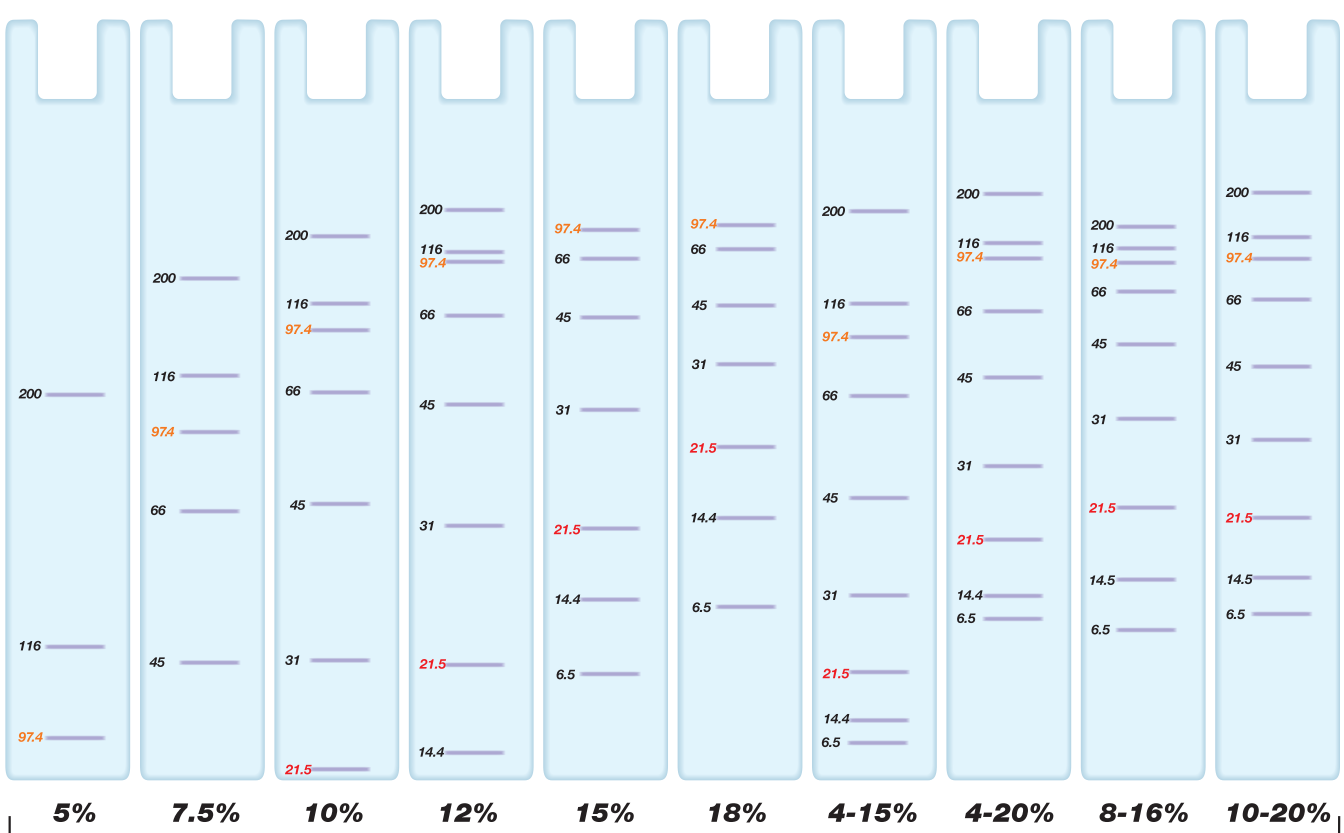
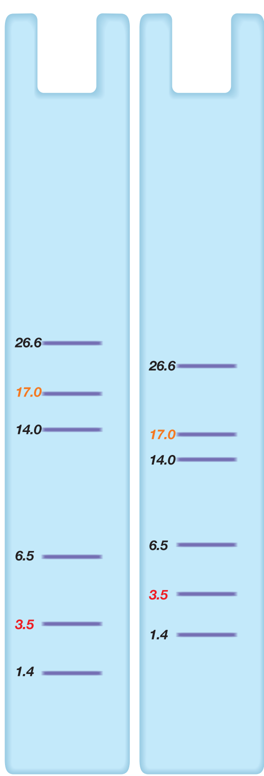


Tris-HCl

Standard protein migration on Tris-HCl Ready Gels. The molecular weight of each band is given in kDa. Migrations are based on the leading dye front being run to the bottom of a gel. Single percentage gels produce the greatest resolution between any two bands that are close in molecular weight, whereas a linear gradient gel allows both high and low molecular weight bands to be visualized on the same gel.

Ordering Information

Gel	Qty	9 Well	10 Well/30 µl	10 Well/50 µl	12 Well	15 Well	2-D/Prep
5%	1	161-1215	161-1210	161-1213	161-1214	161-1211	161-1212
5%	10	161-1057	161-1052	161-1055	161-1056	161-1053	161-1054
5%	20	161-1365	161-1360	161-1363	161-1364	161-1361	161-1362
7.5%	1	161-1190	161-1100	161-1154	161-1172	161-1118	161-1136
7.5%	10	161-0980	161-0900	161-0960	161-0970	161-0930	161-0950
7.5%	20	161-1340	161-1250	161-1304	161-1322	161-1268	161-1286
10%	1	161-1191	161-1101	161-1155	161-1173	161-1119	161-1137
10%	10	161-0987	161-0907	161-0967	161-0977	161-0937	161-0957
10%	20	161-1341	161-1251	161-1305	161-1323	161-1269	161-1287
12%	1	161-1192	161-1102	161-1156	161-1174	161-1120	161-1138
12%	10	161-0981	161-0901	161-0961	161-0971	161-0931	161-0951
12%	20	161-1342	161-1252	161-1306	161-1324	161-1270	161-1288
15%	1	161-1193	161-1103	161-1157	161-1175	161-1121	161-1139
15%	10	161-0988	161-0908	161-0968	161-0978	161-0938	161-0958
15%	20	161-1343	161-1253	161-1307	161-1325	161-1271	161-1289
18%	1	161-1221	161-1216	161-1219	161-1220	161-1217	161-1218
18%	10	161-1063	161-1058	161-1061	161-1062	161-1059	161-1060
18%	20	161-1371	161-1366	161-1369	161-1370	161-1367	161-1368
4-15%	1	161-1194	161-1104	161-1158	161-1176	161-1122	161-1140
4-15%	10	161-0982	161-0902	161-0962	161-0972	161-0932	161-0952
4-15%	20	161-1344	161-1254	161-1308	161-1326	161-1272	161-1290
4-20%	1	161-1195	161-1105	161-1159	161-1177	161-1123	161-1141
4-20%	10	161-0983	161-0903	161-0963	161-0973	161-0933	161-0953
4-20%	20	161-1345	161-1255	161-1309	161-1327	161-1273	161-1291
8-16%	1	161-1227	161-1222	161-1225	161-1226	161-1223	161-1224
8-16%	10	161-1069	161-1064	161-1067	161-1068	161-1065	161-1066
8-16%	20	161-1377	161-1372	161-1375	161-1376	161-1373	161-1374
10-20%	1	161-1196	161-1106	161-1160	161-1178	161-1124	161-1142
10-20%	10	161-0986	161-0906	161-0966	161-0976	161-0936	161-0956
10-20%	20	161-1346	161-1256	161-1310	161-1328	161-1274	161-1292

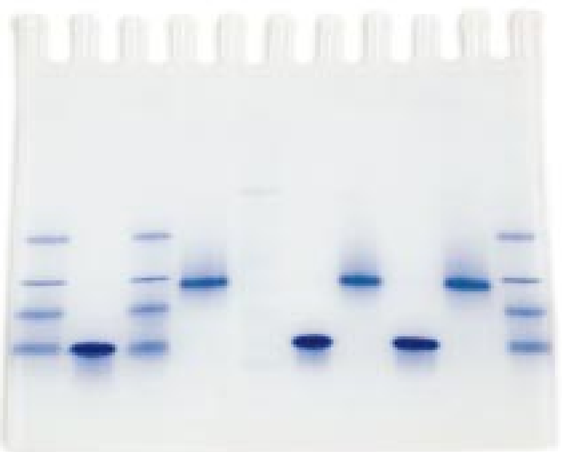


Standard protein migration on Tris-Tricine Ready Gels. The molecular weight of each band is given in kDa. Migrations are based on the leading dye front being run to the bottom of a gel.

Tris-Tricine Ready Gels

Tris-Tricine Ready Gels offer the ideal system for separating peptides and proteins as small as 1,000 daltons. The Tris-Tricine buffer system separates small protein-SDS complexes from SDS micelles, which in most other buffer systems interfere with the separation of small, fast-running proteins. Tricine SDS-PAGE has also proven useful for resolving mixtures of lipopolysaccharides and lipooligosaccharides.

- ***Ideal for separating peptides and proteins as small as 1,000 daltons***
- ***Resolves mixtures of lipopolysaccharides and lipooligosaccharides***



10-20% Tris-Tricine Ready Gel, 10 well, catalog number 161-1108. Samples: lanes 1, 3, 10, Polypeptide SDS-PAGE Standards; lanes 2, 6, 8, Ubiquitin; lanes 4, 7, 9, Ribonuclease A; lane 5, Carboxypeptidase Inhibitor. Stained with Coomassie G-250.

Tip #16

Seeing skewed bands? *Sample salt concentration and excessive power can make proteins run irregularly, causing the middle portion of the band to run faster than the rest (smiling bands or slanted lanes). Localized distortions within the gel can be caused by uneven heat distribution and inadequate heat dissipation.*

Ordering Information

Gel	Qty	9 Well	10 Well/30 µl	10 Well/50 µl	12 Well	15 Well	2-D/Prep
16.5%	1	161-1197	161-1107	161-1161	161-1179	161-1125	161-1143
16.5%	10	161-0948	161-0922	161-0944	161-0946	161-0925	161-0942
16.5%	20	161-1347	161-1257	161-1311	161-1329	161-1275	161-1293
10-20%	1	161-1198	161-1108	161-1162	161-1180	161-1126	161-1144
10-20%	10	161-0949	161-0923	161-0945	161-0947	161-0926	161-0943
10-20%	20	161-1348	161-1258	161-1312	161-1330	161-1276	161-1294

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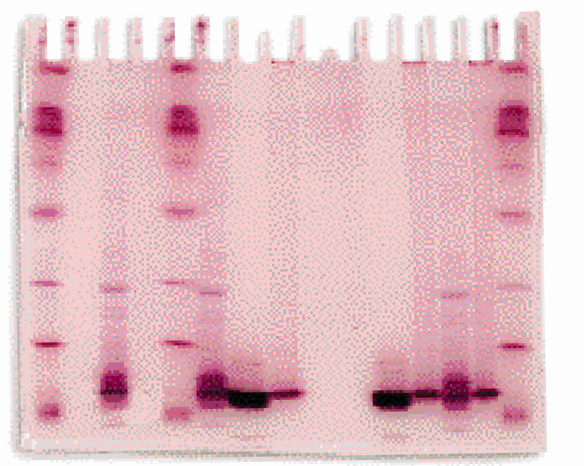
IEF Ready Gels

IEF Ready Gels use an optimized photopolymerization chemistry with riboflavin 5-phosphate which produces IEF gels with a very low abundance of charged species. Using a vertical slab format, sample volumes up to 50 µl can be run in an analytical gel, and up to 450 µl can be loaded onto a preparative gel. The results are easily visualized with premixed Coomassie/Crocein Scarlet IEF stain. IEF Ready Gels also feature a 6-month shelf life.

- ***Sample volumes of 15 µl to 450 µl can be loaded***
- ***6-month shelf life***

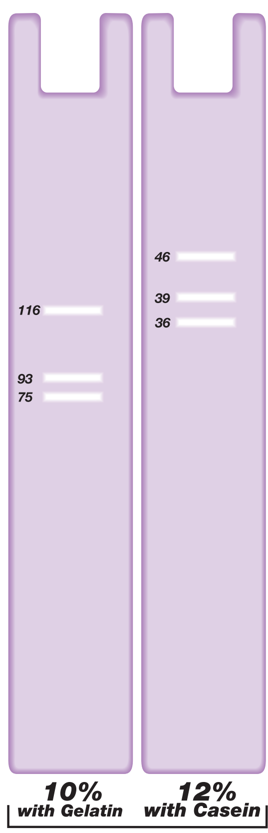


5-8 range IEF Ready Gel, 15 well, catalog number 161-1130. Samples: lanes 1,5,15, IEF Standards; 2, 4, 9, 10, blank; 3, 6, 13, Southern Copperhead Snake Venom; 7, 11, C. Phycocyanin; 8, 12, 14, Skunk Serum. IEF Stain (Coomassie R-250 / Crocein Scarlet).

Standard protein migration on IEF Ready Gels. Each band is given in pI units.

Ordering Information

Gel	Qty	9 Well	10 Well/30 µl	10 Well/50 µl	12 Well	15 Well	2-D/Prep
pH 3-10	1	161-1201	161-1111	161-1165	161-1183	161-1129	161-1147
pH 3-10	10	161-1015	161-1010	161-1013	161-1014	161-1011	161-1012
pH 3-10	20	161-1351	161-1261	161-1315	161-1333	161-1279	161-1297
pH 5-8	1	161-1202	161-1112	161-1166	161-1184	161-1130	161-1148
pH 5-8	10	161-1021	161-1016	161-1019	161-1020	161-1017	161-1018
pH 5-8	20	161-1352	161-1262	161-1316	161-1334	161-1280	161-1298



Standard protein migration on Zymogram Ready Gels. 10% gel loaded with Collagenase F sample. 12% gel loaded with Plasmin sample. The molecular weight of each band is given in kDa. Migrations are based on the leading dye front being run to the bottom of a gel.

Zymogram Ready Gels

Zymogram Ready Gels let you test for proteolytic activity quickly and easily. Gelatin or casein is incorporated into the gel providing a substrate for proteases. A positive result is a clear band in the stained gel. Let the convenience of Ready Gels help expand the ways you analyze proteins.

- ***Test for proteolytic activity quickly and easily***
- ***Adds a new test parameter to the convenience of Ready Gels***

10% Zymogram Ready Gel with Gelatin, 10 well, catalog number 161-1113. Samples: lane 1, blank, lanes 2-10, Collagenase F. Stained with Coomassie Blue R-250.

Ordering Information

Gel	Qty	9 Well	10 Well/30 µl	10 Well/50 µl	12 Well	15 Well
10% Gelatin	1	161-1203	161-1113	161-1167	161-1185	161-1131
10% Gelatin	10	161-1027	161-1022	161-1025	161-1026	161-1023
10% Gelatin	20	161-1353	161-1263	161-1317	161-1335	161-1281
12% Casein	1	161-1204	161-1114	161-1168	161-1186	161-1132
12% Casein	10	161-1033	161-1028	161-1031	161-1032	161-1029
12% Casein	20	161-1354	161-1264	161-1318	161-1336	161-1282

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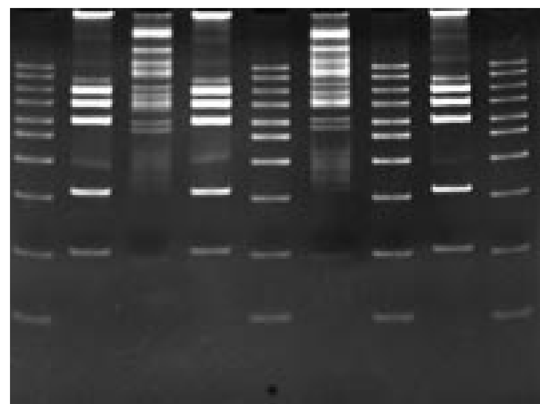
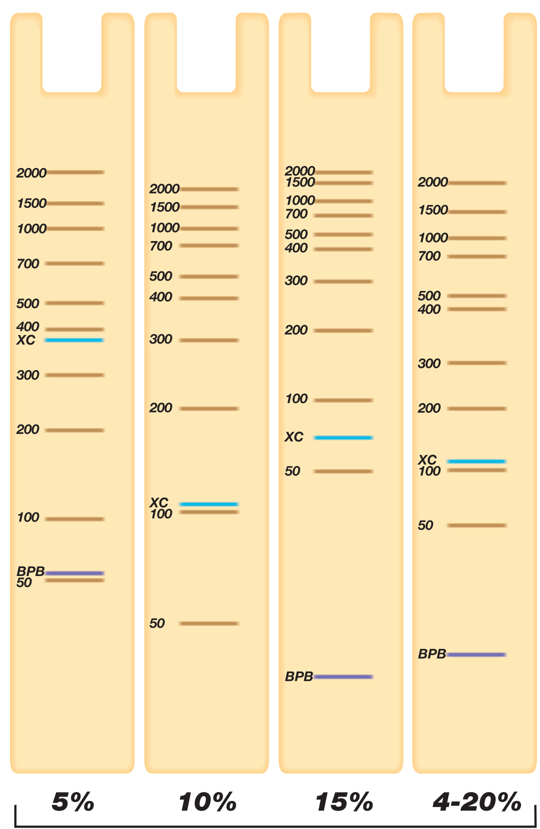
TBE Ready Gels

Uniform Tris-Boric Acid-EDTA (TBE) Ready Gels are prepared for continuous buffer, non disassociating electrophoresis for nucleic acids. TBE Ready Gels provide rapid high resolution separations of nucleic acids from 50–2,000 base pairs. This is ideal for analyzing the purity of PCR fragments, and many DNA and RNA applications. Acrylamide-based TBE Ready Gels provide higher resolution than agarose gels, and require no preparation.

- ***Rapid high resolution separations of nucleic acids from 50–2,000 base pairs***
- ***Ideal for analyzing the purity of PCR fragments***



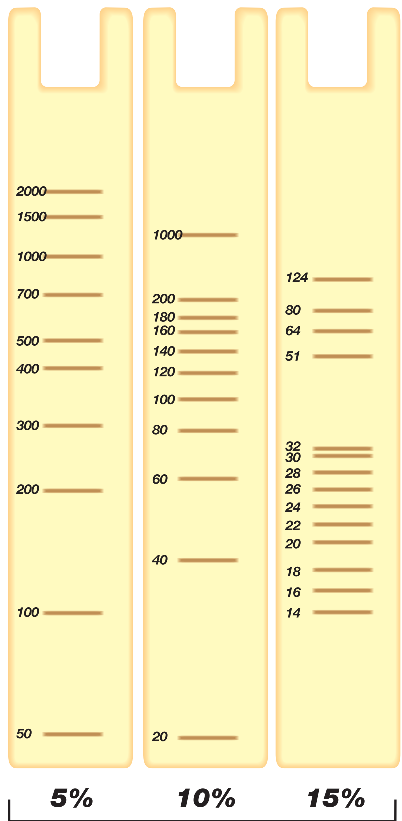
10% TBE Ready Gel, 9 well, catalog number 161-1200. Samples: lanes 1, 5, 7, 9, AmpliSize Standard; lanes 2, 4, 8, 100 bp Molecular Ruler; lanes 3, 6, Low Range Standard pBE322 digest with *Ava*II and *Ava*II/*Eco*R1.



Standard protein migration on TBE Ready Gels. The molecular weight of each band is given in base pairs for TBE gels. The following is the length of time the gels were run as shown in this chart:
TBE 5% = 45 minutes
TBE 10% = 90 minutes
TBE 15% = 105 minutes
TBE 4-20% = 105 minutes
XC = Xylene Cyanol FF
BPB = Bromophenol Blue

Ordering Information

Gel	Qty	9 Well	10 Well/30 µl	10 Well/50 µl	12 Well	15 Well
5%	1	161-1199	161-1109	161-1163	161-1181	161-1127
5%	10	161-0984	161-0904	161-0964	161-0974	161-0934
5%	20	161-1349	161-1259	161-1313	161-1331	161-1277
10%	1	161-1200	161-1110	161-1164	161-1182	161-1128
10%	10	161-0985	161-0905	161-0965	161-0975	161-0935
10%	20	161-1350	161-1260	161-1314	161-1332	161-1278
15%	1	161-1233	161-1228	161-1231	161-1232	161-1229
15%	10	161-1075	161-1070	161-1073	161-1074	161-1071
15%	20	161-1383	161-1378	161-1381	161-1382	161-1379
4-20%	1	161-1239	161-1234	161-1237	161-1238	161-1235
4-20%	10	161-1081	161-1076	161-1079	161-1080	161-1077
4-20%	20	161-1389	161-1384	161-1387	161-1388	161-1385

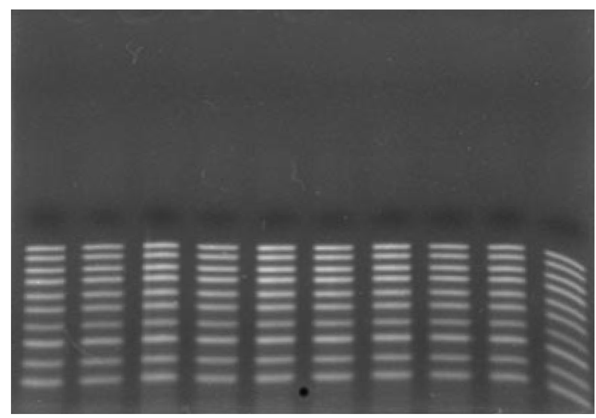


Standard protein migration on TBE-Urea Ready Gels. The molecular weight of each band is given in bases for TBE-Urea gels. The following is the length of time the gels were run as shown in this chart:
TBE-Urea 5% = 70 minutes
TBE-Urea 10% = 90 minutes
TBE-Urea 15% = 40 minutes

TBE-Urea Ready Gels

For denaturing nucleic acid applications, TBE-Urea Ready Gels are cast with 7 M Urea to provide the conditions necessary for denaturing electrophoresis. These gels provide rapid high resolution separations of single strand nucleic acids from 20–2,000 bases.

- ***Rapid, high resolution separations of single strand nucleic acids from 20–2,000 bases***
- ***Useful for oligonucleotide purity analysis, RNAse protection assays, and northern blotting***



15% TBE-Urea Ready Gel, 10 well, catalog number 161-1117. Samples: repeating lanes Oligo Size Standard, 8-32 mer. Ethidium Bromide stain.

Ordering Information

Gel	Qty	9 Well	10 Well/30 µl	12 Well	2-D/Prep
5%	1	161-1205	161-1115	161-1187	161-1051
5%	10	161-1039	161-1034	161-1038	161-1036
5%	20	161-1355	161-1265	161-1337	161-1301
10%	1	161-1206	161-1116	161-1188	161-1052
10%	10	161-1045	161-1040	161-1044	161-1042
10%	20	161-1356	161-1266	161-1338	161-1302
15%	1	161-1207	161-1117	161-1189	161-1053
15%	10	161-1051	161-1046	161-1050	161-1048
15%	20	161-1357	161-1267	161-1339	161-1303

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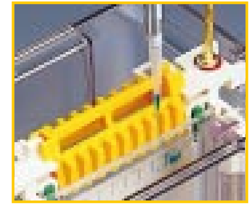
Ready Gel System Equipment

Accelerate your research with the convenient Ready Gel Cell

The Ready Gel Cell is by far the easiest way to run Ready Gels. This industry standard was engineered for exceptional speed, safety, and reliability. The Ready Gel Cell features self alignment pegs, pressure plates, and a pair of soft-touch cam closures for a reliable, leak-proof seal. Heat is dissipated away from the gels so evenly and efficiently, you can run Tris-HCl SDS gels at 200 volts, resulting in a finished gel run in as little as 35 minutes with high quality results. Sample loading is quick and simple, thanks to Bio-Rad's exclusive sample loading guide. It fits neatly in the top of the Ready Gel Cell, guiding your pipette to the right well every time.

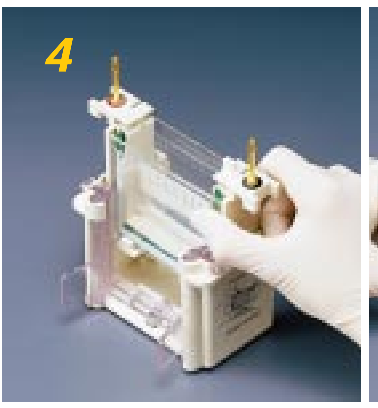


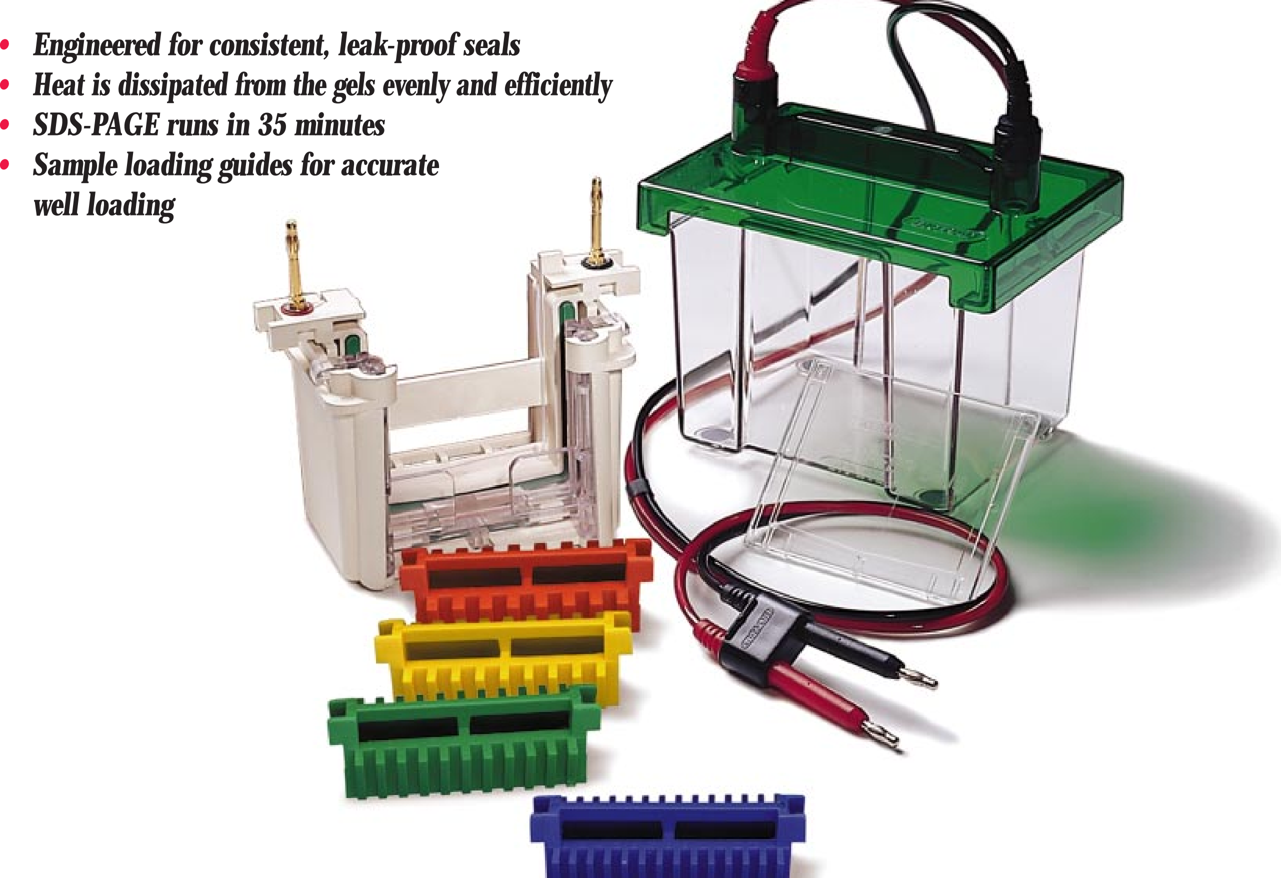
The Ready Gel Cell uses the same tank and lid as the Mini-PROTEAN II cell and the Mini Trans-Blot cell, so you get maximum value from the Bio-Rad systems you may already own.

- ***Engineered for consistent, leak-proof seals***
- ***Heat is dissipated from the gels evenly and efficiently***
- ***SDS-PAGE runs in 35 minutes***
- ***Sample loading guides for accurate well loading***

Tip #17

Preparing to run Ready Gels in the Ready Gel Cell takes only seconds.





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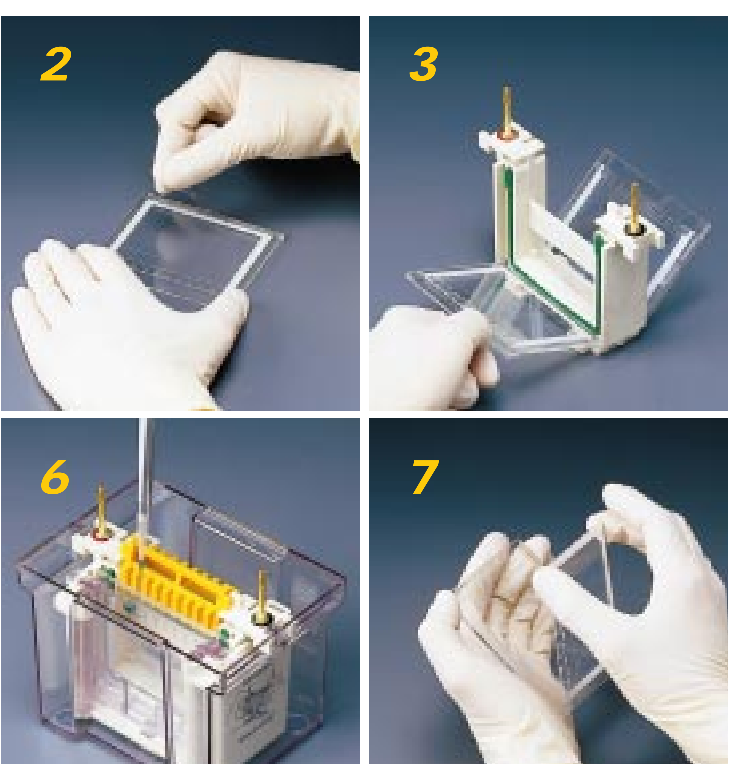

1. Use the key knife or a razor blade to cut the tape at the bottom of the gel along the black "cut here" line. It is helpful to cut all the way to the edge of the cassette where the pull tab begins. 2. Pull the tape tab along the cut line, up from the cassette and at an angle towards the comb end of the gel.
3. Place the gel cassette in the Electrode Assembly. When placed correctly, the gel will rest at a 45° angle against the assembly. Push the gel up into place to form a tight, leakproof seal. 4. When both gels or one gel and the buffer dam are in place, put the assembled electrode assembly into the Clamping Frame. 5. Using both index fingers, push down gently on the electrode assembly to seat in place. At the same time, use your thumbs to close the clamping frame's cam levers and lock the gels in place. 6. Pipet the samples into the wells using the appropriate sample loading guide. Run gel. Disassemble. 7. Open the cassettes by lifting the shorter glass plate from one corner with your thumbs.

Ordering Information

Catalog No.	Product Description
165-3125	Ready Gel Cell, includes electrode assembly, clamping frame, mini tank with power cables, mini cell buffer dam, one 10-well and one 15-well Sample Loading Guide, and instructions
165-3126	Ready Gel Cell Module, includes electrode assembly, clamping frame, mini cell buffer dam, one 10-well and one 15-well Sample Loading Guide, and instructions
165-3156	Ready Gel Cell and Mini Trans-Blot Module, includes Ready Gel Cell and Mini Trans-Blot module with modular electrode assembly, 2 gel holder cassettes, 4 fiber pads, Bio-Ice cooling unit, and instructions
170-3930	Mini Trans-Blot Electrophoretic Transfer Cell, includes 2 gel holder cassettes, 4 fiber pads, modular electrode assembly, Bio-Ice cooling unit, lower buffer chamber, lid with cables, and instructions
170-3935	Mini Trans-Blot Module, without lower buffer chamber and lid

Ready Gel Cell Accessories

165-3130	Ready Gel Cell Buffer Dam, 2
161-0990	Empty Ready Gel cassettes, 10 each
161-0999	9 well combs, 10 each
161-0994	10 well combs, 10 each
161-0997	10 well/50 µl combs, 10 each
161-0998	12 well combs, 10 each
161-0995	15 well combs, 10 each
161-0996	2-D/Prep combs, 10 each
161-0992	Key Knife, 1
161-0993	Ready Gel Instruction Manual, 1
165-3121	Sample Loading Guide, 9 well (red), 1
165-3146	Sample Loading Guide, 10 well (yellow), 1
165-3203	Sample Loading Guide, 12 well (green), 1
165-3132	Sample Loading Guide, 15 well (blue), 1

Mini Trans-Blot Cell for Western Transfers of Ready Gels

The Mini Trans-Blot Cell provides rapid, high quality blotting of Ready Gels. The modular design of the cassette holder fits into the Ready Gel Cell tank, providing maximum flexibility in transfers, and minimizing the amount of tank equipment in the lab. The tank blotting process ensures complete transfer of proteins for quantitative analysis.

- ***Fast transfer of two Ready Gels in one hour***
- ***Alternative low voltage, overnight transfer***
- ***Specially molded design protects platinum wire from breaking***
- ***Cassette holder fits into the Ready Gel Cell tank for easy set up***

Ready Gel Cell and Mini Trans-Blot Module

EQUIPMENT: POWER SUPPLIES

Bio-Rad's power supplies are designed to match the right power parameters to your technique. Use the following Power Supply Selection Guide to identify the right power supply for your application.

Power Supply Selection Guide by Electrophoretic Method

Method	Power Supply	Recommended Apparatus	Type of Ready Gel	Typical Power Conditions Per 2 Gels (C=Constant)				
				BEGIN RUN		END RUN		
				Approx. Volt	mA	Approx. Volt	mA	Typical Run Time
Laemmli (SDS)	PowerPac 300	Ready Gel Cell	Tris-HCl	200 (C)	100	200 (C)	60	35 min.
Laemmli (SDS) Polypeptide	PowerPac 300	Ready Gel Cell	Tris-Tricine	100 (C)	65	100 (C)	35	100 min.
Ornstein-Davis, Native Discontinuous	PowerPac 300	Ready Gel Cell	Tris-HCl	200 (C)	100	200 (C)	60	35 min.
Isoelectric Focusing; Vertical Ready Gel	PowerPac 1000	Ready Gel Cell	IEF	Stepwise: 100 (C) 250 (C) 500 (C)	5-25 5-25 5-25	- - -	- - -	1 hour* 1 hour* 30 min.*
Protease Analysis	PowerPac 300	Ready Gel Cell	Zymogram	100 (C)	25	100 (C)	12	80-100 min.
DNA / RNA	PowerPac 300	Ready Gel Cell	TBE	100 (C)	26	100 (C)	22	45-105 min.
Denaturing DNA / RNA	PowerPac 300	Ready Gel Cell	TBE-Urea	200 (C)	30	200 (C)	20	40-70 min.
Western Blotting	PowerPac 200	Mini Trans-Blot cell	2 Gels	100 (C)	250	100 (C)	350	1 hr
Semi-Dry Protein Blotting	PowerPac 200	Trans-Blot SD cell	2 Gels	15 (C)	500	15 (C)	200	15 min.

* Total run time 2.5 hours.

Ordering Information

Catalog No.	Product Description
165-5050	PowerPac 300 Power Supply,† 100/120 V
165-5051	PowerPac 300 Power Supply,† 220/240 V
165-5052	PowerPac 200 Power Supply, 100/120 V
165-5053	PowerPac 200 Power Supply, 220/240 V
165-5054	PowerPac 1000 Power Supply, 100/120 V
165-5055	PowerPac 1000 Power Supply, 220/240 V
Power Supply Accessories	
165-5061	PowerPac Adaptor†
165-5062	PowerPac Shelf

†The PowerPac Adapter is required when connecting non-IEC certified electrophoresis cells with banana plug handles ≤ 26 mm long (excluding the metal portion) to the PowerPac 200 or 300 Power Supply.

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PowerPac 200 Power Supply
for all blotting applications

With 2.0 ampere capability, the PowerPac 200 is the perfect supply for all blotting applications including tank blotting, semi-dry blotting, and high current nucleic acid transfers from acrylamide gels. With constant voltage or current, and timer control, this unit is also suitable for everyday mini-vertical and submarine electrophoresis usage.

Tip #18

Power conditions for multiple cells. *If running constant voltage, 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. Be sure the current limit is set high enough to permit this additive function. Also make certain you're using a power supply that can accommodate this additive current.*

- ***200V, 2.0A, 200W***
- ***Best for all blotting applications***
- ***Timer control lets you walk away during a run***

Tip #19

What is IEC1010-1? *IEC is the International Electrotechnical Commission, which was formed to create an international standard for electrical equipment. This standard sets a very stringent level of safety for electrical equipment. By complying with IEC1010-1 standards, Bio-Rad manufactures equipment that meets high standards for laboratory safety. In many cases, this means a longer electrical contact on the apparatus. There are adapters available through Bio-Rad to retrofit older equipment.*

PowerPac 300 Power Supply
for the Ready Gel Cell and Sub-Cells

The PowerPac 300 lets you run four Ready Gel Cells at once with constant voltage or current, and continuous or timed runs. The complete Ready Gel system includes the Ready Gel Cell and this power supply.

- ***300V, 400mA, 75W***
- ***Best for mini-vertical and submarine gel electrophoresis***
- ***Timer control lets you walk away during a run***

PowerPac 1000 Power Supply
for 2-D and general purpose use

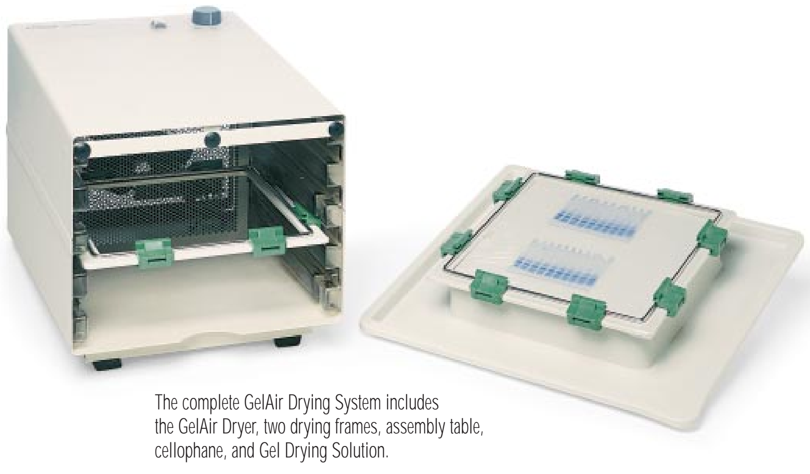
The current, higher voltage, and step-programmability capability of the PowerPac 1000 make this suitable for IEF, SDS or native-PAGE, and is the ideal choice for 2-D electrophoresis.

- ***1000V, 500mA, 250W***
- ***Best for IEF, 2-D electrophoresis, and SDS or native-PAGE***
- ***Delivers current to microamp level for IEF gels***

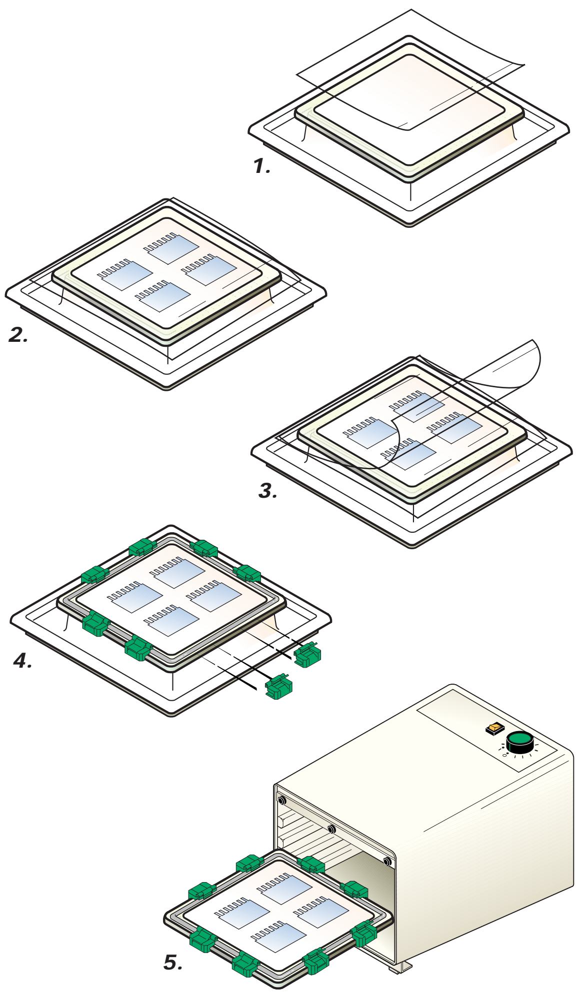
GelAir Drying System

The GelAir Drying System is perfect for drying Ready Gels since it's fast, easy, and efficient. Gels dry between two sheets of cellophane, resulting in perfectly clear, publication quality gels ideal for densitometry. Each drying frame holds one to four Ready Gels and mini-submarine agarose gels, or one large format (20 x 20 cm) gel. The GelAir dryer holds up to four drying frames.

- ***Up to 16 Ready Gels dry in less than 60 minutes***
- ***No waiting for a complete drying cycle; the GelAir dryer accepts gels at any time***
- ***No vacuum pump required, so gel drying is maintenance free***



The complete GelAir Drying System includes the GelAir Dryer, two drying frames, assembly table, cellophane, and Gel Drying Solution.



Drying gels with GelAir is easy. 1. Place a sheet of cellophane on the assembly table; 2. Lay a gel on the cellophane; 3. Place a second sheet of cellophane over the gel; 4. Clamp the drying frames together; 5. Slide the drying frame into the dryer.

Gel Drying Solution – liquid insurance against cracking

The Gel Drying Solution is a pretreatment for polyacrylamide gels which helps prevent cracking in gels during air drying.

- ***Quick 10-minute equilibration for clean, consistent results***

Tip #20

Avoid warping frames. *The GelAir Dryer is engineered for fast, efficient drying of gels. The fan operates with or without heat, and requires a minimum of 10 cm of clearance in back of the unit for adequate ventilation. Inadequate clearance can cause heat to build up and warp the drying frames.*

Ordering Information

Catalog No.	Product Description
165-1771	GelAir Drying System, 115 V, 60 Hz,
165-1772	GelAir Drying System, 230 V, 50 Hz
165-1777	GelAir Dryer, 115 V, 60 Hz
165-1778	GelAir Dryer, 230 V, 50 Hz
165-1775	GelAir Drying Frames, includes 2 frames and 16 clamps
165-1776	GelAir Assembly Table
165-1779	GelAir Cellophane Support, 50 precut sheets
165-1780	GelAir Drying Frame Clamps, 8
161-0752	Gel Drying Solution, 1 L

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Ready Gel Reagents

Premixed Sample and Running Buffers

Another way to save preparation time in your electrophoresis runs is to use liquid concentrate buffers. Premixed Sample and Running Buffers are made with Bio-Rad's electrophoresis purity reagents and are quality controlled to ensure reproducible results.

- ***Wide selection of buffers provides for virtually any protocol***
- ***Concentrated Premixed Sample Buffers allow use with liquid and lyophilized samples***

Ordering Information

Catalog No.	Product Description	Quantity
Premixed Buffers		
161-0732	10x Tris/Glycine/SDS	1 L
161-0755	10x Tris/Glycine/SDS	6 x 1 L
161-0734	10x Tris/Glycine	1 L
161-0757	10x Tris/Glycine	6 x 1 L
161-0744	10x Tris/Tricine/SDS	1 L
161-0760	10x Tris/Tricine/SDS	6 x 1 L
161-0733	10x Tris/Boric Acid/EDTA	1 L
161-0756	10x Tris/Boric Acid/EDTA	6 x 1 L
161-0741	10x TBE Extended Range	1 L
161-0758	10x TBE Extended Range	6 x 1 L
161-0743	50x Tris/Acetic Acid/EDTA	1 L
161-0759	50x Tris/Acetic Acid/EDTA	6 x 1 L
161-0761	10x IEF Anode Buffer	250 ml
161-0762	10x IEF Cathode Buffer	250 ml
161-0765	10x Zymogram Renaturing Buffer	250 ml
161-0766	10x Zymogram Development Buffer	250 ml
Premixed Sample Buffers		
161-0737	Laemmli Sample Buffer	30 ml
161-0738	Native Sample Buffer*	30 ml
161-0739	Tricine Sample Buffer	30 ml
161-0763	IEF Sample Buffer	30 ml
161-0764	Zymogram Sample Buffer 30 ml	
161-0767	TBE Sample Buffer	30 ml

*Store at 4 °C. All other reagents store at room temperature.

Premixed Running Buffer Composition

	Description (1x Buffer Concentration)
Tris/Glycine/SDS	25 mM Tris 192 mM Glycine 0.1% SDS
Tris/Glycine	25 mM Tris 192 mM Glycine
Tris/Tricine/SDS	100 mM Tris 100 mM Glycine 0.1% SDS
Tris/Boric Acid/EDTA	89 mM Tris 89 mM Boric Acid 2 mM EDTA
TBE Extended Range*	130 mM Tris, 4 5 mM Boric Acid 2.5 mM EDTA
Tris/Acetic Acid/EDTA	40 mM Tris 20 mM Acetic Acid 1 mM EDTA
10 x IEF Cathode	20 mM Lysine, 20 mM Arginine
10 x IEF Anode	7 mM Phosphoric Acid
Zymogram Renaturation	2.5% Triton X-100
Zymogram Development	50 mM Tris-HCl (pH 7.5) 200 mM NaCl 5 mM CaCl2 0.02% Brij-35

*Extends buffer capacity for longer DNA sequencing runs.

Sample Buffer Composition

	Description (1x Buffer Concentration)
Laemmli	62.5 mM Tris-HCl 2% SDS 25% Glycerol 0.01% Bromophenol Blue
Native	62.5 mM Tris-HCl 40% Glycerol 0.01% Bromophenol Blue
Tricine	200 mM Tris-HCl 2% SDS 40% Glycerol 0.04% Coomassie G-250
IEF	50% Glycerol
Zymogram	62.5 mM Tris-HCl 4% SD 25% Glycerol 0.01% Bromophenol Blue
TBE	1x TBE 50% Glycerol 0.005% Bromophenol Blue 0.025% Xylene Cyanol
Ficoll	6% Ficoll 0.5x TBE 0.005% Bromophenol Blue 0.025% Xylene Cyanol 3.5 M Urea

All buffers are made with 18 M ohm water.

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Prot/Elec Pipet Tips for gel loading

- ***Fits easily between vertical slab gel plates down to 0.75 mm***
- ***Combine Prot/Elec Tips and the Ready Gel Cell Loading Guides for exceptionally easy sample loading***

Prot/Elec Pipet Tip

Buffer Reagents and Detergents

For protocols that require a different buffer or concentration than the premixed buffers available, Bio-Rad provides the finest electrophoresis reagents from Tris to premixed SDS solutions.

Hand Casting Reagents

Bio-Rad continues to supply the reagents that helped make electrophoresis the well-characterized, reliable system it is today. If there are special gel requirements you can't find in the Ready Gel products, these reagents provide a time-tested means for pouring your own gel in empty Ready Gel cassettes to be run in the Ready Gel Cell.

Ordering Information

Catalog No.	Product Description	Quantity
Prot/Elec Pipet Tips		
223-9915	Prot/Elec Tips, bulk pack, plastic bag in dust free box	1,000
223-9917	Prot/Elec Tips, racked, 200 per rack	1,000
Buffer Reagents and Detergents		
161-0719	Tris	1 kg
161-0751	Boric Acid	1 kg
161-0729	EDTA	500 g
161-0718	Glycine	1 kg
161-0731	Urea	1 kg
161-0720	Sucrose	1 kg
161-0611	Dithiothreitol,	5 g
161-0710	2-mercaptoethanol[1]	25 ml
161-0712	Tricine	100 g
161-0416	SDS Solution 10% (w/v)	250 ml
161-0418	SDS Solution 20% (w/v)	1 L
161-0301	SDS (Sodium dodecyl sulfate)	100 g
161-0407	Triton X-100 Detergent	500 ml
170-6531	Tween 20 EIA Grade	100 ml
161-0752	Gel Drying Solution	1L
Acrylamide Powder		
161-0100	Acrylamide[1], 99.9%	100 g
161-0101	Acrylamide[1], 99.9%	500 g
Premixed Acrylamide/Bis Powders[1]		
161-0121	Acrylamide/Bis, 29:1	30 g
161-0122	Acrylamide/Bis, 37.5:1	30 g
Acrylamide Solutions[1,2]		
161-0156	30% Acrylamide/Bis Solution, 29:1	500 ml
161-0158	30% Acrylamide/Bis Solution, 37.5:1	500 ml
161-0144	40% Acrylamide/Bis Solution, 19:1	500 ml
161-0148	40% Acrylamide/Bis Solution, 37.5:1	500 ml
161-0140	40% Acrylamide Solution	500 ml
161-0142	2% Bis Solution	500 ml
Crosslinkers		
161-0200	Bis[1]	5 g
161-0142	2% Bis Solution[1,2]	500 ml
Catalysts		
161-0800	TEMED[1,3]	5 ml
161-0700	Ammonium Persulfate[1,3]	10 g
PAGE Reagent Starter Kit[1]		
161-5100	PAGE Reagent Starter Kit, includes Acrylamide, 100 g; Bis, 5 g; TEMED, 5 ml; Ammonium Persulfate, 10 g	

1. Hazardous shipping charges may apply.
2. Store at 4 °C.
3. For longer shelf life, store desiccated at room temperature.

All other reagents should be stored at room temperature, dry, and away from direct sunlight.

Stains Comparison

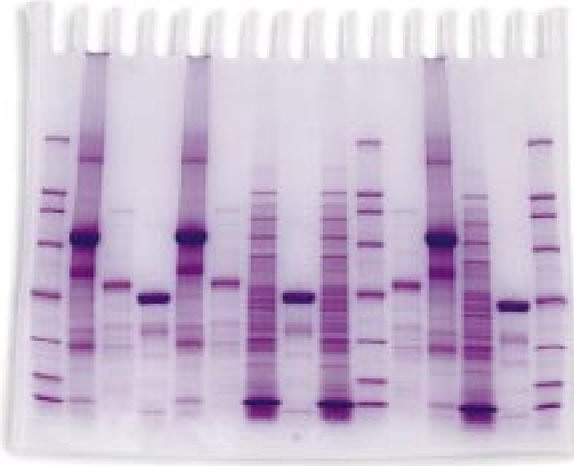
Stain	Sensitivity	Advantages	Application
Coomassie Blue	0.1–1 µg/band	Simple, fast, consistent	General Protein Stain
IEF Stain	0.1–1 µg/band	Simple, fast, consistent	IEF gel stain
Zinc Stain	10–100 ng/band	Simple, fast, reversible; subsequent electro-elution or blotting is possible	SDS-PAGE
SYPRO Orange	2–10 ng/band	Highly sensitive fluorescent stain; subsequent transfer and blotting is possible	SDS-PAGE
Silver Stain/Silver Stain Plus	1–10 ng/band	Highly sensitive; can also stain nucleic acids	Protein and Nucleic Acids
Ethidium Bromide	0.1-10 ng/band	Premixed solution minimizes handling	Nucleic Acids
Radiant Red	10 ng RNA	30-minute stain with no destain	RNA

As with many aspects of laboratory research, there are numerous ways to detect proteins in a Ready Gel. The different methods provide varying levels of sensitivity, cost, time in processing, and post-stain alternatives. Bio-Rad's wide selection of stains offers many alternatives, and you can be assured each one will deliver results with speed and convenience.

Premixed Coomassie Brilliant Blue R-250 Stain and Destain

Premixed solutions make this popular stain easier and more convenient to use than ever.

- ***No powders to weigh or solutions to filter***
- ***Stain and destain are packaged ready to use***



4-15% Tris-HCl Ready Gel, 15 well, Coomassie R-250 stain.

Protocol Overview

1. Use enough stain solution to completely cover the gel(s). Stain at room temperature with rocking or agitation for 30-45 minutes.
2. Place gels in destain solution for 45 minutes agitation, repeat with fresh destain solution for 45 minutes.
3. An alternative to #2 is to add a sponge to the tray with approximately 2x volume of destain and leave agitating overnight.
4. Remove gel from destain solution when desired signal to background is obtained.

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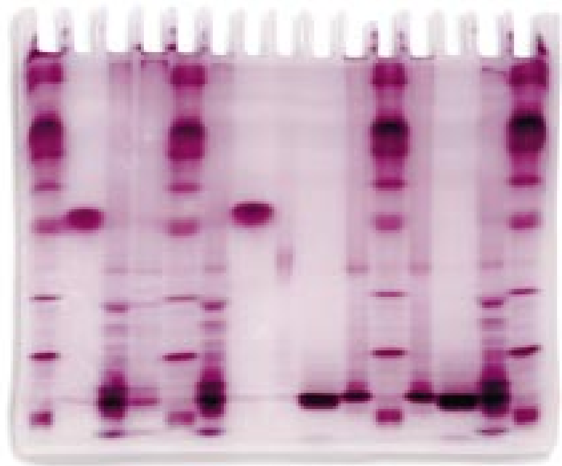
IEF Gel Stain, Premixed Coomassie R-250/Crocein Scarlet

Provides quick and easy visualization of IEF Ready Gels with a brilliant red color. Use the Coomassie Destain for destaining steps.

- ***Eliminates the time and mess involved with making the solution from powder***
- ***Consistent quality staining from gel to gel***



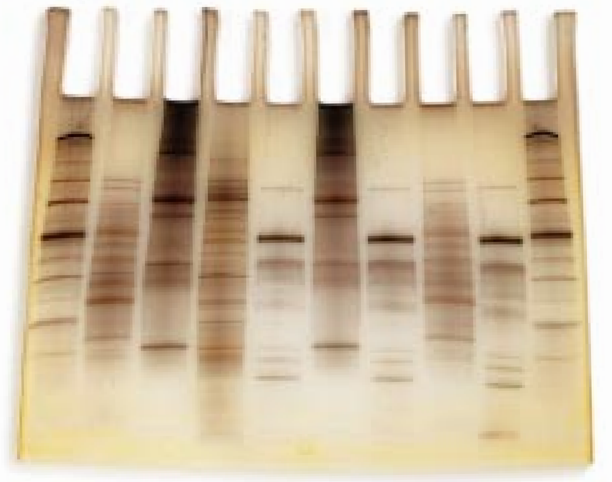
3-10 range IEF Ready Gel, 15 well, IEF stain (Coomassie / Crocein Scarlet).

Protocol Overview
1. Stain IEF Ready Gel for 45 minutes agitating.
2. Destain for 2-3 hours in multiple changes of Destaining Solution.

Silver Stain Plus Kit

Achieve the sensitivity once only available to the most highly skilled staining experts with this modified chemistry

- ***Simple, reproducible method***
- ***Low background, high sensitivity results***



10-20%, Tris-HCl Ready Gel, 50 µl well, Silver Stain Plus stain.

Protocol Overview
1. Fix - 20 minutes in Fixative Enhancer Solution.
2. Rinse - 2 x 10 minutes in ddi water.
3. Stain and Develop - 20 minutes Staining Solution (prepare within 5 minutes of use).
4. Stop - 15 minutes in 5% acetic acid stop solution.

Bio-Rad Silver Stain Kit

The time-tested version of the silver stain is derived from the original method of Merril, *et al.*

- ***Stains any protein sample***
- ***Ideal for polyacrylamide IEF gels, polysaccharides, and highly glycosylated proteins***

Protocol Overview
1. Fix - 2 x 15 minutes in acid/alcohol with shaking.
2. Oxidizer - 5 minutes.
3. Wash - 10 minutes.
4. Silver reagents - 20 minutes.
5. Wash - 1 to 30 seconds.
6. Developer - 1 to 10 minutes.
7. Stop - 5% acetic acid solution.

Ordering Information

Catalog No.	Product Description
161-0435	Coomassie R-250 Staining Kit[1], includes 1 L Stain, 2 x 1 L Destain
161-0436	Coomassie Brilliant Blue R-250 Staining Solution, 1 L
161-0438	Coomassie Brilliant Blue R-250 Destaining Solution, 1 L
161-0434	IEF Staining Solution[1]
161-0449	Silver Stain Plus Kit[1], for 40 Ready Gels
161-0443	Bio-Rad Silver Stain Kit[1,3], for 24 Ready Gels

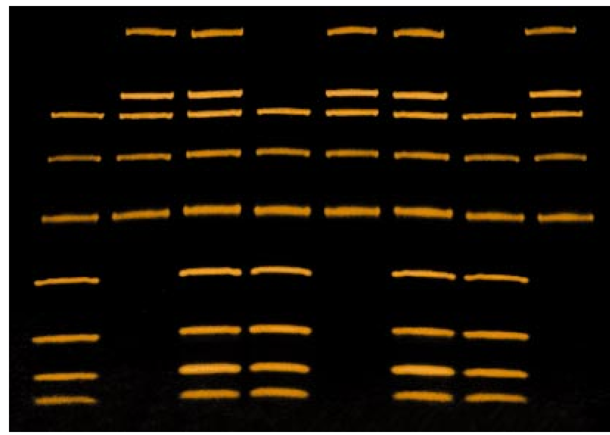
1. Hazardous shipping charges may apply.
2. Must be used together.
3. Store at 4 °C.

BIO-RAD

SYPRO Orange Fluorescent Protein Stain

SYPRO Orange brings the power of fluorescence to your protein gel. Sensitivity approaching Silver Stain can be achieved with digital imaging equipment, such as Bio-Rad's Gel Doc 1000 System.

- ***Fast 30-minute incubation in the stain***
- ***Proteins can be transferred without destaining and without inhibiting western blotting***



4-20%, Tris-HCl Ready Gel, 10 well, SYPRO Orange fluorescent stain.

Protocol Overview

1. Dilute - SYPRO Orange concentrate 1:5,000 in 7.5% acetic acid in a plastic tray.
2. Stain - Add gel, cover, and shake for 30 minutes.
3. Rinse - 30 to 60 seconds in 7.5% acetic acid.
4. Image - Photograph on a UV transilluminator or digitally image the gel.

Tip #21

For lower background with SYPRO Orange. *Try a running buffer with 0.05% SDS instead of the normal 0.1% SDS (10x Tris-Glycine plus 5 g dry SDS makes Tris-Glycine / 0.05% SDS). This is close to the least amount of SDS practical for good electrophoresis results, so additions should be made accurately.*

Zinc Stain and Destain Kit

Zinc stains the gel around the proteins but does not directly stain the proteins. The results appear as clear protein bands within a white stained gel.

- ***Stains in just 10 minutes***
- ***Ideal for detection of proteins followed by recovery from the gel***

4-20%, Tris-HCl Ready Gel, 9 well, Zinc stain visualized on a black background.

Protocol Overview

1. Dilute - Solutions A and B to 1x.
2. Stain - 10 minutes in solution A.
3. Stain - 30 seconds to 1 minute in solution B.
4. Stop - Transfer gel to ddi water at desired stain intensity.

Tip #22

Mechanism of SYPRO Orange and Zinc staining. *SYPRO Orange and Zinc stains work because there is a higher concentration of SDS on the protein than in the background gel. SYPRO Orange is attracted to higher concentrations of SDS forming a positive detection signal, while Zinc works because it is repelled by SDS and deposits in the gel matrix around the proteins, forming a negative stain.*

Ordering Information

Catalog No.	Product Description
170-3120	SYPRO Orange Protein Stain, 500 µl, 50-100 Ready Gels
161-0440	Zinc Stain & Destain Kit, stains 25-50 Ready Gels
161-0441	Imidazole[1], 10x Zinc Stain Solution A, 125 ml
161-0442	Zinc Sulfate[1], 10x Zinc Stain Solution B, 125 ml

1. Must be used together.

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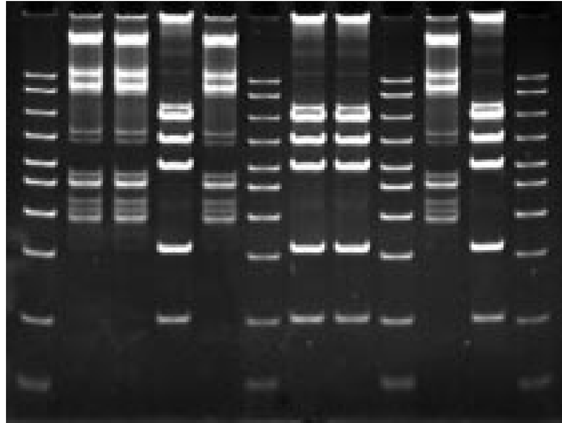
Ethidium Bromide Solution

The most common nucleic acid stain works equally well on TBE acrylamide gels.

- ***Eliminate exposure to hazardous powder with premixed solution***
- ***Visualize the gel on a transilluminator or an imaging instrument such as the Fluor-S MultiImager system***



5%, TBE Ready Gel, 12 well, Ethidium Bromide stain.

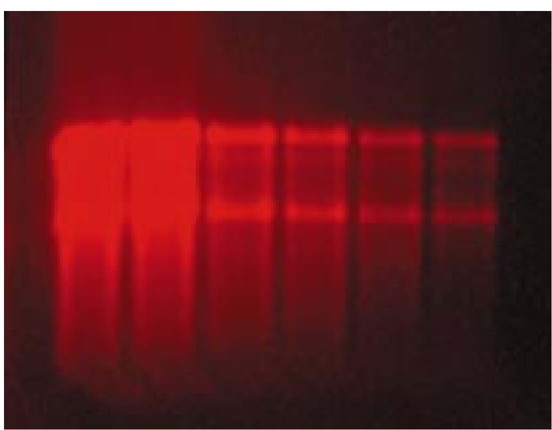
Protocol Overview

1. Stain - Add 5 to 10 µl to running buffer.
2. Alternative - gel can be incubated in stain after electrophoresis.
3. Visualize - transilluminator or imaging equipment.

Radiant Red RNA Stain

Radiant Red RNA stain is a highly sensitive fluorescent stain for visualizing RNA in denaturing agarose gels, TBE, and TBE-Urea Ready Gels.

- ***Single 30-minute staining without pre-soaking or destaining***
- ***Excited by standard 302 nm wavelength***



Radiant Red Fluorescent RNA Stain.

Protocol Overview

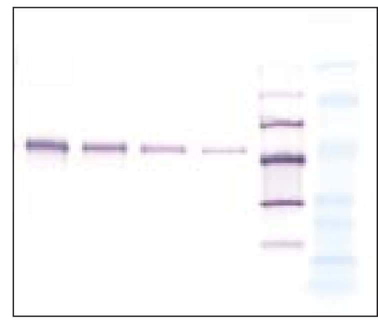
1. Dilute - 1:1,000 in running buffer.
2. Stain - 30 minutes with shaking.
3. Visualize - transilluminator or imaging equipment.

Ordering Information

Catalog No.	Product Description
161-0433	Ethidium Bromide Solution, 10 ml, 10 mg/ml
161-0430	Ethidium Bromide Tablets, 10 x 11 mg
170-3122	Radiant Red Fluorescent RNA Stain, 10 ml

REAGENTS: GLYCOPROTEIN ANALYSIS

Bio-Rad's glycoprotein analysis products encompass a system of precast gels, electrophoresis buffers, analysis kits, and imaging equipment designed to answer fundamental but powerful questions about the N-linked glycoprotein structure. The following table provides some guidelines for choosing the best method to achieve your goals in carbohydrate analysis using electrophoretic methods.



Specific labeling and detection of glycoproteins using the Immun-Blot kit for glycoprotein detection.

Carbohydrate Analysis Methods

Question	Goal	Glycoprotein Product	Analysis Method
Is my protein glycosylated?	Detect presence or absence of glycoprotein	Immun-Blot Kit for Glycoprotein Detection	BCIP/NBT color development on western blot.
Are there oligosaccharides on the protein?	Cleave oligosaccharides from protein, look at mobility shift	Enzymatic Deglycosylation Kit	Enzymatic cleavage of oligosaccharides. Gel stain for mobility shift.
What is the monosaccharide composition of the carbohydrate?	Qualitatively identify the monosaccharides in the composition	Monosaccharide Compositional Analysis Kit	Hydrolysis of the monosaccharides. Fluorescent labeling. Electrophorese and analyze by imager.
What kind of oligosaccharides are on the protein?	Profile carbohydrate by specific release of either N- or O-linked oligosaccharides	Oligosaccharide Profiling Kits; N-linked or O-linked	Enzymatic release of oligosaccharide. Fluorescent labeling of oligosaccharide. Electrophorese and analyze by imager.
What is the monosaccharide sequence of the oligosaccharide?	Determine the sequence of the N-linked oligosaccharide	N-linked Oligosaccharide Sequencing Kit	Specific enzymatic cleavage of monosaccharides. Electrophorese and analyze

Ordering Information

Catalog No.	Product Description
170-6490	Immun-Blot Kit for Glycoprotein Detection, includes periodate, biotin hydrazide, blocking reagent, bisulfate, streptavidin-alkaline phosphatase, color development reagents, positive control, biotinylated markers, SDS-PAGE markers
170-6500	Enzymatic Deglycosylation Kit, includes O-Glycosidase DS, NANase II, PNGase F, glycoprotein control, and reaction buffers
170-6508	Deglycosylation Enhancement Kit, includes GALase III, HEXase I, and 2x PNGase F reaction buffer
Enzymes	
170-6880	HEXase I, 42 U/ml, 40 µl
170-6881	O-Glycosidase ds, 1 U/ml, 40 µl
170-6882	NANase II, 5 U/ml, 40 µl
170-6883	PNGase F, 2.5 U/ml, 40 µl
170-6513	GALase III, 1.5 U/ml, 40 µl
Analysis Kits	
170-6811	Monosaccharide Compositional Analysis Kit, includes hydrolysis reagents, AMAC fluorophore, precast gels, premixed buffers, and standards.
170-6501	N-Linked Oligosaccharide Profiling Kit, includes PNGase F, ANTS fluorophore, precast gels, premixed buffers, and standards.
170-6815	O-Linked Oligosaccharide Profiling Kit, includes hydrazine, ANTS fluorophore, precast gels, premixed buffers, and standards.

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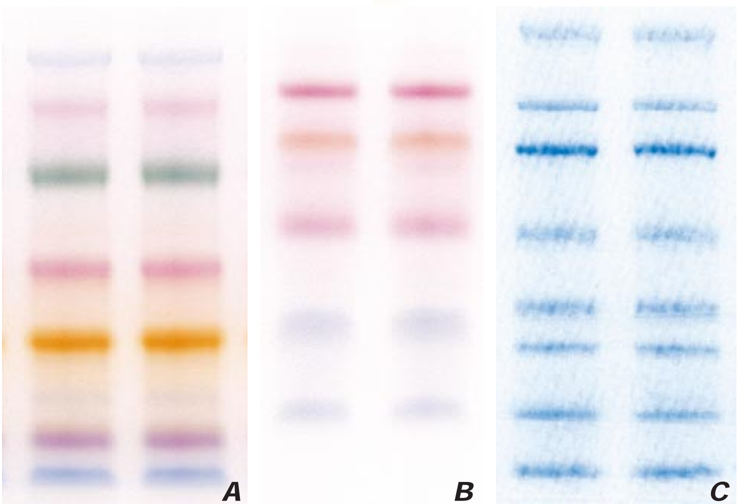
Standards
accelerate
your
RESEARCH
PARAFILM "M"
PARAFILM "M"
PARAFILM "M"
PARAFILM "M"

Standards are an integral part of every experiment in helping identify and characterize the molecules separated in an electrophoretic gel. Standards allow the calibration of protein molecular weights and number of DNA base pairs, the determination of isoelectric points, and analysis of 2-D gels. Standards also furnish a general check of the electrophoresis run and are a check for making valid gel-to-gel comparisons.

Calibrated molecular weights. *Bio-Rad calibrates every lot of prestained standards, and includes these estimated molecular weights with each product. However, the prestained standards should not be used to calibrate an unknown protein on the gel, since that means estimating a molecular weight from another estimation.*



A. Kaleidoscope Prestained Standards and **B. Kaleidoscope Polypeptide Standards** are individually colored proteins or polypeptides blended together to give a spectrum of colors on Western blots or SDS-PAGE gels; **C. Prestained SDS-PAGE Standards** are a consistent blue color, and are available in High, Low, and Broad molecular weights.

Prestained Protein Standards

Prestained standards are manufactured with a colored dye added to the protein making them visible in a gel or on a blot for easy orientation. Kaleidoscope Prestained Standards are multi-colored for easy band recognition. Prestained SDS-PAGE Standards are dyed a uniform blue color. Both are excellent prestained standards with the following features:

- ***Easy assessment of blotting efficiency***
- ***Continuous monitoring of electrophoresis separation***
- ***Ready to use; no dilution necessary***
- ***Molecular weight of each protein is calibrated and reported with every lot***

Protocol Overview

1. Heat to 40 °C for one minute to dissolve any solids. Do not overheat.
2. Ready Gel load: 10 µl to visualize on the gel, 5 µl for a blot.

Kaleidoscope Prestained Protein Standards

Kaleidoscope standards are mixtures of protein standards uniquely prestained with colored dyes to provide instant band recognition.

- ***Kaleidoscope Prestained Standards cover the range of 6 - 200 kDa***
- ***Kaleidoscope Polypeptide Standards cover the range of 3 - 40 kDa***

Prestained SDS-PAGE Standards

The original blue stained proteins are available in high, low, and broad molecular weight ranges.

- ***Excellent for monitoring the results of a western***

Ordering Information

Catalog No.	Product Description	Quantity	Applications Per Vial
161-0324	Kaleidoscope Prestained Standards	500 µl	100
161-0325	Kaleidoscope Polypeptide Standards	500 µl	100
161-0305	Prestained SDS-PAGE Standards, low range	500 µl	100
161-0309	Prestained SDS-PAGE Standards, high range	500 µl	100
161-0318	Prestained SDS-PAGE Standards, broad range	500 µl	100

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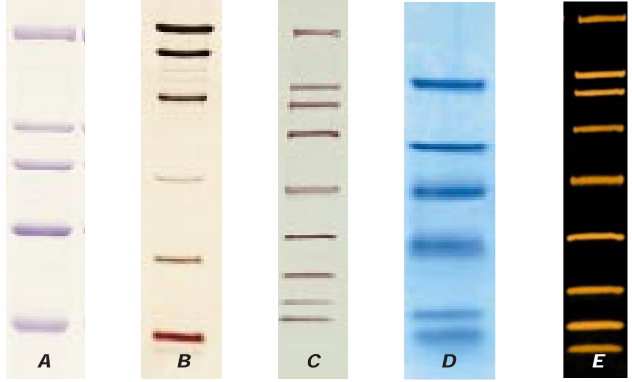
Bio-Rad's molecular weight standards are mixtures of purified proteins visualized by staining in the gel simultaneously with sample protein staining. By plotting a calibration curve with the standards, accurate molecular weight determinations of sample proteins can be made on any SDS polyacrylamide gel.

- ***Several standard ranges cover virtually every gel and stain***
- ***Pre-blended in a stable buffer for fast, easy preparation and use***
- ***Glycerol buffer eliminates freeze/thaw degradation and the need to aliquot***

Protocol Overview
1. Dilute MW standard 1:20 in SDS reducing sample buffer.
2. Add fresh 2-mercaptoethanol.
3. Heat for five minutes at 95 °C.



SDS-PAGE Standards add to the analytical power of Ready Gels. A. High range SDS-PAGE Standards run on a 7.5% Ready Gel and stained with Coomassie blue R-250 dye. **B.** Low range Silver Stain SDS-PAGE Standards run on a 12% gel and stained with Bio-Rad's Silver Stain Kit. **C.** Broad range Biotinylated SDS-PAGE standards run on a 4–20% gradient gel, blotted to nitrocellulose, and detected with Avidin-AP. **D.** Polypeptide SDS-PAGE Standards run on a 16.5% tricine gel and stained with Coomassie blue G-250 dye. **E.** Broad range SDS-PAGE standards for SYPRO Orange staining run on a 4–20% gel and stained with SYPRO Orange.

Composition of SDS-PAGE, Silver Stain, and SYPRO Orange Fluorescent Standards

Protein	MW (Daltons)	Polypeptide	Low	High	Broad
Myosin	200,000	-	-	X	X
b-galactosidase	116,250	-	-	X	X
Phosphorylase B	97,400	-	X	X	X
Bovine Serum albumin	66,200	-	X	X	X
Ovalbumin	45,000	-	X	X	X
Carbonic anhydrase	31,000	-	X	-	X
Triosephosphate isomerase	26,625	X	-	-	-
Trypsin inhibitor	21,500	-	X	-	X
Myoglobin	16,950	X	-	-	-
∝-Lactalbumin	14,437	X	-	-	-
Lysozyme	14,400	-	X	-	X
Aprotinin	6,500	X	-	-	X
Insulin, b chain, oxidized	3,496	X	-	-	-
Bacitracin	1,423	X	-	-	-

Biotinylated Standards

Biotinylated standards are an excellent tool for molecular weight calibration on western blots. See the Protein Blotting section, page 60, for details.

Ordering Information

Catalog No.	Product Description	Quantity	Applications Per Vial
161-0326	Polypeptide SDS-PAGE Standards	200 µl	800
161-0304	SDS-PAGE Standards, low range	200 µl	800
161-0303	SDS-PAGE Standards, high range	200 µl	800
161-0317	SDS-PAGE Standards, broad range	200 µl	800
161-0314	Silver Stain SDS-PAGE Standards, low range	200 µl	800
161-0315	Silver Stain SDS-PAGE Standards, high range	200 µl	800
161-0330	SDS-PAGE Standards for SYPRO Orange, low range	200 µl	800
161-0331	SDS-PAGE Standards for SYPRO Orange, high range	200 µl	800
161-0332	SDS-PAGE Standards for SYPRO Orange, broad range	200 µl	800

IEF Standards

IEF Standards permit dependable and reproducible pI calibration in IEF Ready Gels. IEF Standards consist of a mixture of nine native proteins with isoelectric points ranging from 4.45 to 9.6.

Tip #24

Native, non-denaturing conditions: *IEF Standards are meant for use in non-denaturing conditions. If these standards are used with urea, the band pattern will be changed, significantly shifted, and the natural color will be lost, so these standards are not recommended for urea IEF gels. For calibration of IEF tube gels containing urea, use 2-D SDS-PAGE standards.*

- ***Five of the nine proteins are naturally colored***
- ***No reconstitution or dilution required***

Protocol Overview
1. No dilution or pretreatment necessary.
2A. Coomassie stained gels, use 5 µl / lane.
2B. Silver stained gels, use 0.5 µl (or 5 µl of a 1:10 dilution).

Constituent Proteins of IEF Standards

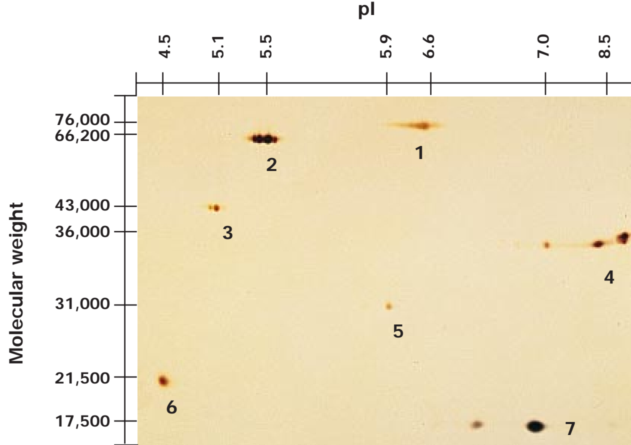
Protein	Color	pI
Cytochrome c	Red	9.6
Lentil lectin (3 bands)		7.8, 8.0, 8.2
Human hemoglobin C	Red	7.50
Human hemoglobin A	Red	7.10
Equine myoglobin (2 bands)	Brown	7.00
Human carbonic anhydrase		6.50
Bovine carbonic anhydrase		6.00
ß-Lactoglobulin B		5.10
Phycocyanin (3 bands)	Blue	4.45, 4.65,

2-D SDS-PAGE Standards

These unique protein standards calibrate the pI and molecular weight of proteins in 2-D SDS-PAGE applications.

- ***Seven reduced, denatured proteins***
- ***Marker for gel to gel reproducibility and analysis***
- ***No dilution required***

Protocol Overview
1. No dilution required.
2. Vortex after thawing to resuspend.
3A. Coomassie stained Ready Gels, use 2.5 µl.
3B. Silver stained Ready Gels, use 0.5 - 2.5 µl.



Two-dimensional electrophoretic pattern of the 2-D SDS-PAGE standards.

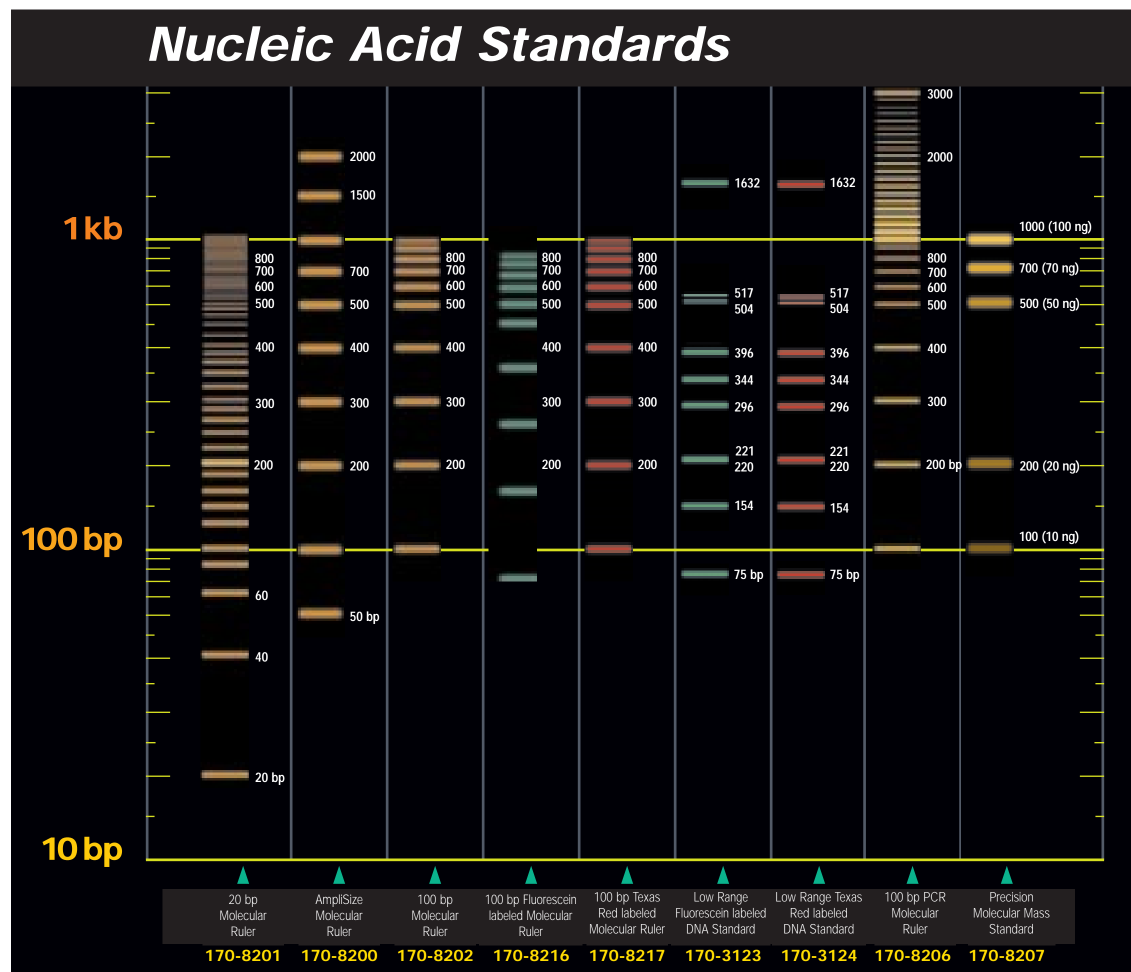
Constituent Proteins of 2-D SDS-PAGE Standards

Protein	pI	MW (Daltons)	Reference
Hen egg white conalbumin	6.0, 6.3, 6.6	76,000	1
Bovine serum albumin	5.4, 5.5, 5.6	66,200	2, pI empirically determined
Bovine muscle actin	5.0, 5.1	43,000	pI empirically determined
Rabbit muscle GAPDH	8.3, 8.5	36,000	3
Bovine carbonic anhydrase	5.9, 6.0	31,000	4, 5, 6
Soybean trypsin inhibitor	4.5	21,500	7, 8
Myoglobin	7.0	17,500	9

References: 1. *Experientia*, 34, 849 (1978). 2. Brown, J. R., *Fed. Proc.*, **34,** 591 (1975). 3. Smith, C. M. and Velick, S. F., *J. Biol. Chem.*, **247,** 273 (1972). 4. Davis, R. P., Carbonic Anhydrase, in *The Enzymes*, Vol. V, p 545 (Boyer, P. D. ed) Academic Press, New York (1971). 5. Ashworth, R. B. and Spencer, T .E., *Arch. Biochem. Biophys.*, **142,** 122 (1971). 6. Jonsson, M. and Petterson, E., *Acta. Chem. Scand.*, **22,** 712 (1968). 7. Wu, Y. V. and Scheraga, H. A., *Biochem.*, **1,** 698 (1962). 8. Hall, J. A., *Anal. Biochem.*, **31,** 437

Ordering Information

Catalog No.	Product Description	Quantity	Applications Per Vial
161-0310	IEF Standards, pI range 4.45-9.6	250 µl	50
161-0320	2-D SDS-PAGE Standards	500 µl	200



Bio-Rad's high quality DNA standards are essential tools for the molecular biologist and a perfect complement to TBE Ready Gels.

Molecular Rulers

Molecular Rulers provide the precision and quality demanded by today's molecular biologists. Molecular Rulers progress by precise and easily calculable increments, making them ideal for densitometry or image analysis.

Ordering Information

Catalog No.	Product Description	Applications	Storage
170-8200	AmpliSize Molecular Ruler	50 lanes	4 °C
170-8201	20 bp Molecular Ruler	100 lanes	4 °C
170-8202	100 bp Molecular Ruler	100 lanes	4 °C
170-8206	100 bp PCR Molecular Ruler	100 lanes	4 °C
170-3123	Low Range Fluorescein labeled DNA Standard	125 lanes	4 °C
170-3124	Low Range Texas Red labeled DNA Standard	125 lanes	4 °C
170-8216	100 bp Fluorescein labeled Molecular Ruler	125 lanes	4 °C
170-8217	100 bp Texas Red labeled Molecular Ruler	125 lanes	4 °C
170-3465	Low Range Standard, 88-1,746 bp,	50 lanes	4 °C
170-8207	Precision Molecular Mass Standard 100-1,000 bp, 10-100 ng	100 lanes	4 °C

Fluorescent DNA Standards

Fluorescent labeled standards are ideal for fast, accurate imaging and precise, multiplexed molecular weight determinations. Perform multiplexing by mixing fluorescently labeled DNA standards and complementary fluorescently end-labeled DNA samples.

Full Range DNA Standards

Full Range DNA standards are conventional restriction digests of plasmid or viral DNA, tested for proper concentration and mobility to provide a reliable control for gel-to-gel variability.

Precision Molecular Mass Standard

The Precision Molecular Mass Standard is ideal for accurate quantitation of DNA in gels. The standard contains five coordinated bands of decreasing mass ranging from 100 ng to 10 ng.

Western Blotting

accelerate *your* RESEARCH

The transfer of proteins from a gel can be accomplished in several different ways. There are specific equipment and technique differences, along with considerations regarding the expected outcome of the transfer. Two common transfer techniques are tank blotting, and semi-dry blotting. The following section describes the Mini Trans-Blot cell for tank blotting, and the Trans-Blot SD for semi-dry blotting.

Tip #25

Gel and membrane setup. *For standard basic transfers, the gel should be on the cathode (black) side of the sandwich, and the membrane on the anode (red) side. For acidic transfers, the gel and membrane positions should be reversed.*

** Incorrect configuration will run the proteins out of the gel into the transfer buffer, and the membrane will be blank.*

Comparison of Tank and Semi-Dry Cells

	Mini Trans-Blot Cell (tank blotting)	Trans-Blot SD Cell (semi-dry blotting)
Electrode Distance Buffer Capacity	4 cm 450 ml	Determined by gel thickness Minimal; ~250 ml per experiment
Power Conditions	Begin Run: 100 V (C), 250 mA, 1 hour End Run: 100 V (C), 350 mA, 1 hour	Begin Run: 15 V (C), 500 mA, 15 minutes End Run: 15 V (C), 200 mA
Transfer Times	1 hour for Ready Gels. Extended transfers (up to 24 hours) possible	Rapid transfers; extended transfers not possible because of buffer depletion
Multiple Gel Capacity	2 Ready Gels	4 Ready Gels
Transfer Results	Quantitative transfers of wide range of molecular weight proteins	High throughput transfers with optimized transfer conditions

Mini Trans-Blot Electrophoretic Transfer Cell

The Mini Trans-Blot cell provides rapid, high quality blotting of Ready Gels. The modular design of the cassette holder fits into the Ready Gel Cell tank, providing maximum flexibility in transfers, and minimizing the amount of tank equipment in the lab. The tank blotting process ensures complete transfer of proteins for quantitative analysis.

- ***Fast transfer of two Ready Gels in one hour***
- ***Alternative low voltage, overnight transfer***
- ***Specially molded design protects platinum wire from breaking***
- ***Cassette holder fits into the Ready Gel Cell tank for easy set up***

Tip #26

Semi-dry protein range. *Because the semi-dry blotting cell has a limited buffer reservoir, this method is best used with fairly well optimized transfer systems. For proteins that move slowly due to size or pI, the result may be incomplete transfer if the buffer is not optimized.*

Ordering Information

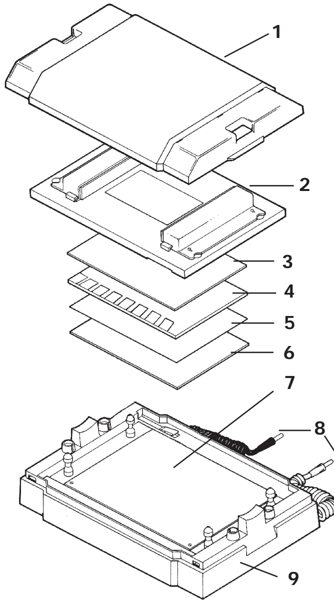
Catalog No.	Product Description
170-3930	Mini Trans-Blot Electrophoretic Transfer Cell, includes 2 gel holder cassettes, 4 fiber pads, modular electrode assembly, Bio-Ice cooling unit, lower buffer chamber, lid with cables, and instructions
170-3935	Mini Trans-Blot Module, without lower buffer chamber and lid
170-3940	Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell*, 100/120 V
170-3949	Trans-Blot SD System, 220/240 V

*The Trans-Blot SD cell requires the use of a microprocessor controlled power supply.
**Blot Absorbent Filter Paper (Extra Thick) is recommended for all blotting applications using the Trans-Blot SD semi-dry transfer cell. See page 57.

Trans-Blot SD Semi-Dry Transfer Cell

The Trans-Blot SD semi-dry transfer cell ensures fast, efficient blotting. The extra thick filter paper in the gel sandwich acts as the buffer reservoir, saving you both time and buffer. The high field strength generated by the close proximity of the plate electrodes completes a transfer from Ready Gels in just 15–30 minutes.

- ***Ideal for fast, routine, qualitative blotting***
- ***Convenient design transfers large or mini gels***
- ***Corrosion-proof platinum and stainless steel electrodes***
- ***Simple set up in just minutes***



An exploded view of the Trans-Blot SD cell. 1. Safety lid. 2. Cathode assembly with latches. 3. Filter paper. 4. Gel. 5. Membrane. 6. Filter paper. 7. Spring-loaded anode platform, mounted on four guide posts. 8. Power cables. 9. Base.

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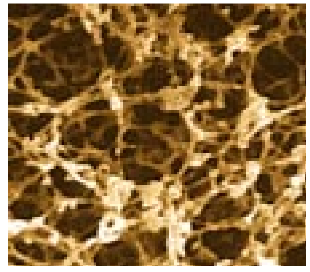
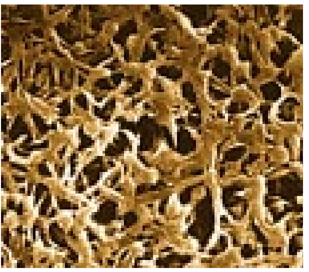
Bio-Rad offers a convenient and comprehensive line of blotting membranes. All protein blotting membranes are sold in rolls or in precut sheets; the 7 x 8.4 cm size fits Ready Gels. Several different membranes are offered since they possess unique characteristics that will benefit various blotting applications. A brief comparison is provided.



Nitrocellulose 4000x microscopic photo.



PVDF 4000x microscopic photo.

Membrane Selection

Membrane	Protein Binding Capacity (µg/cm²)	Features and Applications	Wetting Instructions
Nitrocellulose, 0.45 µ	80-100	Lowest background. Best in tank blotting.	Aqueous transfer buffer.
Nitrocellulose, 0.2 µ	80-100	Binds low MW (<15 kDa). Good for semi-dry blotting.	Aqueous transfer buffer.
Supported Nitrocellulose, 0.45 µ	80-100	Support layer for resistance to cracking and tearing.	Aqueous transfer buffer.
Supported Nitrocellulose, 0.2 µ	80-100	Support layer for resistance to cracking and tearing.	Aqueous transfer buffer.
Immun-Blot PVDF, 0.2 µ onds.	150-160	Low background for blot detection. High strength. Good protein retention with semi-dry and tank blotting.	100% methanol for a few sec- Equilibrate in transfer buffer until it can be easily submerged.
Sequi-Blot PVDF, 0.2 µ onds.	170-200	Maximum binding for protein sequencing,	100% methanol for a few sec-

Ordering Information

Catalog No.	Product Description
Membranes	
162-0145	Nitrocellulose, 0.45 µ, 10 sheets, 7 x 8.4 cm
162-0115	Nitrocellulose, 0.45 µ, 1 roll, 33 cm x 3 m
162-0146	Nitrocellulose, 0.2 µ, 10 sheets, 7 x 8.4 cm
162-0112	Nitrocellulose, 0.2 µ, 1 roll, 33 cm x 3 m
162-0090	Supported Nitrocellulose, 0.45 µ, 10 sheets, 7 x 8.4 cm
162-0094	Supported Nitrocellulose, 0.45 µ, 1 roll, 30 cm x 3 m
162-0095	Supported Nitrocellulose, 0.2 µ, 10 sheets, 7 x 8.4 cm
162-0097	Supported Nitrocellulose, 0.2 µ, 1 roll, 30 cm x 3 m
162-0174	Immun-Blot PVDF membrane, 0.2 µ, 10 sheets, 7 x 8.4 cm
162-0177	Immun-Blot PVDF membrane, 0.2 µ, 1 roll, 26 cm x 3.3 m
162-0186	Sequi-Blot PVDF membrane, 0.2 µ, 10 sheets, 7 x 8.4 cm
162-0184	Sequi-Blot PVDF membrane, 0.2 µ, 1 roll, 26 cm x 3.3 m
Blotting Paper	
162-0118	Thin Blot Filter Paper, 1 roll, 33 cm x 3 m
170-3932	Thick Blot Filter Paper, 50 sheets, 7.5x10 cm
170-3965	Extra Thick Blot Paper, 60 sheets, 7.5 x 10 cm

Tip #27

Improving transfer of larger proteins. *SDS contributes to protein elution out of the gel, but tends to inhibit binding to the membrane. Methanol increases binding of the protein to the membrane, but shrinks pores in the gel, reducing elution. Addition of up to 0.1% SDS to the transfer buffer, or reducing the methanol to 0%, may increase the transfer of proteins. In general, nitrocellulose membranes bind protein more efficiently in the presence of methanol. PVDF membrane does not require methanol, but binding is not reduced in the presence of methanol. Adjusting these parameters can increase the transfer efficiency of your particular protein sample.*

Reagent Selection Guide

Activating Enzyme	Detection Sensitivity	Substrate (result)	Product Options	Advantages	Disadvantages
Colorimetric Horseradish Peroxidase (HRP)	500 pg.	4CN (purple color)	Liquid Substrate, Immun-Blot Kits	Fast color development, inexpensive, low background.	Results fade over time, azide inhibits enzyme activity.
	100 pg.	Opti-4CN (purple color)	Opti-4CN Substrate, Detection Kits	High sensitivity, color does not fade, low background.	More expensive than 4CN.
	5 pg.	Amplified Opti-4CN (purple color)	Amplified Opti-4CN Substrate, Detection Kits	Best sensitivity available, no extra materials (such as X-ray film) needed.	More steps than unamplified protocol.
Colorimetric Alkaline Phosphatase (AP)	100 pg.	BCIP/NBT (purple color)	Liquid Substrate, Immun-Blot Kits	Sensitive, stable storage of data, multiple antigen detection.	Detects endogenous phosphatase activity.
Chemiluminescent Alkaline Phosphatase (AP)	10 pg.	Immun-Star chemiluminescent substrate (X-ray film)	Detection Kits, Substrate Kits	Long lasting light signal, short exposure times, multiple exposures possible.	Film required for visualization of data.

Colorimetric Detection Systems

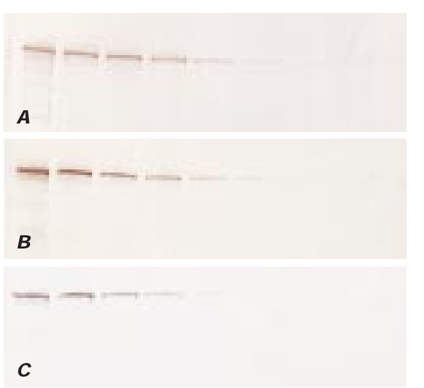
Western blot detection substrates and conjugates offer numerous options for detection sensitivity and data output. Colorimetric options include Amplified Opti-4CN kits for highest detection sensitivity available, premixed liquid Opti-4CN kits, and 4CN and BCIP/NBT as premixed reagents or complete Immun-Blot assay kits.

Opti-4CN Kits; Amplified and Regular Kits

- ***Amplified Opti-4CN kits provide sensitivity equal to or better than chemiluminscence, detection down to 5 pg***
- ***Opti-4CN substrate provides excellent sensitivity and low background results***
- ***Substrate Kits contain substrate reagents; Detection Kits add an HRP conjugated antibody***

Colorimetric Substrates and Kits

- ***Premixed Conjugate Substrates supply solutions of the most popular blotting substrates, BCIP/NBT and 4CN***
- ***Immun-Blot Kits provide all blotting reagents in a convenient kit for application to your primary antibody***



A. 4CN and B. Opti-4CN substrate: Two identical 10% Ready Gels were loaded with a dilution series of human transferrin. Left to right, protein load: 128 ng, 64 ng, 32 ng, 16 ng, 8 ng, 4 ng, 2 ng, 1 ng, 500 pg. **C. Amplified Opti-4CN substrate:** Same gel as above with left to right protein load: 16 ng, 8 ng, 4 ng, 2 ng, 1 ng, 500 pg, 250 pg, 125 pg, 62.5 pg.

Tip #28

Ease of colorimetric development. *Colorimetric development happens as you watch the blot in solution. You can stop the reaction any time you feel the signal to noise is right.*

Ordering Information

Product Description	Substrate Kit Cat. No.	Goat anti Rabbit-HRP Cat. No.	Goat anti Mouse-HRP Cat. No.
Opti-4CN Kits	170-8235	170-8236	170-8237
Amplified Opti-4CN Kits	170-8238	170-8239	170-8240

Ordering Information

Product Description	AP - BCIP/NBT Cat. No.	HRP - 4CN Cat. No.
Premixed Substrate Kit	170-6432	170-6431
Immun-Blot Goat Anti-Rabbit IgG (H+L) Kit	170-6460	170-6463
Immun-Blot Goat Anti-Mouse IgG (H + L) Kit	170-6461	170-6464
Immun-Blot Goat Anti-Human IgG (H + L) Kit	170-6462	170-6465
Immun-Blot Protein A Kit	—	170-6466
Immun-Blot Protein G Kit	—	170-6467

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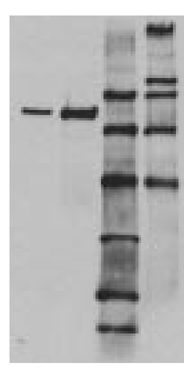
Immun-Star Chemiluminescent System

- ***Fast, sensitive results on nitrocellulose or PVDF membrane blots***
- ***Light signal continues 24 hours after initial activation***
- ***Maximum flexibility in obtaining data***



Left to right: **1:2,000 and 1:200 dilutions of human transferrin;** low range and high range biotinylated standards. Detected with Immun-Star substrate and enhancer on nitrocellulose. Exposure time 30 seconds on film.

Immun-Star Kit Components

Description	Substrate	Enhancer	Antibody	TBS	Tween	Blocker
GAM Detection Kit [1]	X	X	X	—	—	—
GAR Detection Kit [1]	X	X	X	—	—	—
Substrate Pack	X	X	—	—	—	—
Substrate only [1]	X	—	—	—	—	—
GAM Intro Kit [2]	X	X	X	X	X	X
GAR Intro Kit [2]	X	X	X	X	X	X
Blotting Reagents Pack [3]	—	—	—	X	X	X

Immun-Star substrate features CDP-Star technology.
1. All items except Intro Kits cover 2,500 cm^2 of membrane.
2. Intro Kits provide enough of all reagents for 8 mini-blots.
3. Combine the Blotting Reagents Pack with a detection kit to form a complete blotting system.

Ordering Information

Catalog No.	Description
Immun-Star Kits	
170-5010	GAM Detection Kit
170-5011	GAR Detection Kit
170-5012	Substrate Pack
170-5018	Substrate only
170-5013	GAM Intro Kit
170-5014	GAR Intro Kit
170-5015	Blotting Reagents Pack
Blotting Reagents	
170-6537	Gelatin, EIA grade, 200 g
170-6404	Blotting Grade Blocker, non-fat dry milk, 300 g
170-6531	Tween-20, EIA grade, 100 ml
170-6435	Premixed Tris-Buffered Saline, 10x, 1 L
Total Protein Stains	
170-6517	Enhanced Colloidal Gold Total Protein Detection Kit
170-6527	Colloidal Gold Total Protein Stain, 500 ml
161-0402	Amido Black 10B, 25 g
161-0400	Coomassie Blue R-250, 10 g

Blotting Conjugates Ordering Information

Product Description	AP Cat. No.	HRP Cat. No.
Goat Anti-Rabbit IgG (H + L)	170-6518	170-6515
Goat Anti-Mouse IgG (H + L)	170-6520	170-6516
Goat Anti-Human IgG (H + L)	170-6521	172-1050
Protein A	—	170-6522**
Protein G	—	170-6425**
Avidin	170-6533	170-6528
Streptavidin	170-3554*	—

* AP, 0.5 ml. ** HRP, 1 ml. Individual conjugates contain 1 ml AP or 2 ml HRP, except as noted.

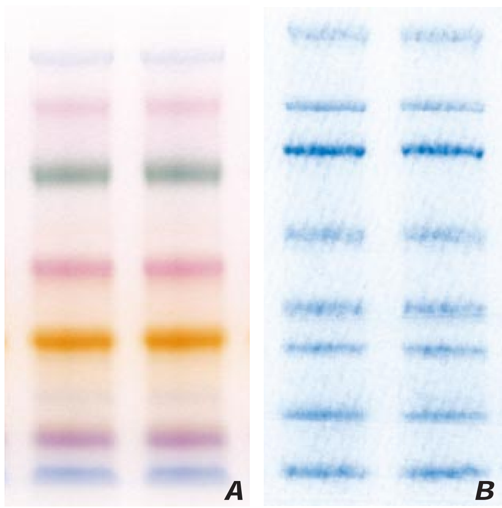
Tip #29

Membrane selection for Immun-Star kits. *The enhancer is used on nitrocellulose blots, but in most cases is not used on PVDF blots. Of all PVDF's available, Immun-Blot PVDF provides the best signal-to-noise results.*

Prestained Standards

Prestained standards are manufactured with a colored dye added to the protein making them visible on blots for easy orientation.

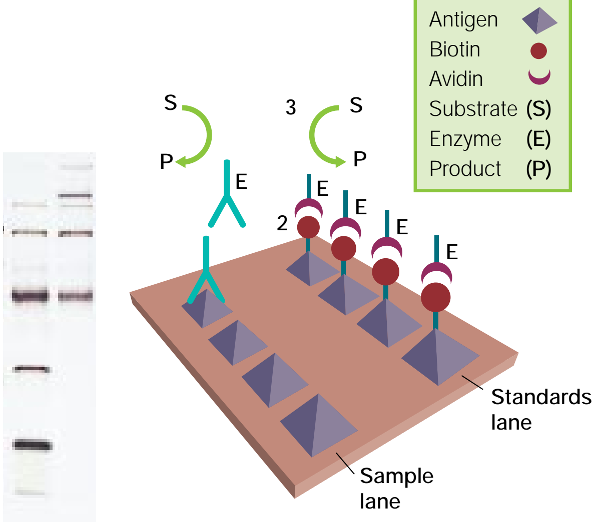
- ***Easy assessment of blotting efficiency***
- ***Kaleidoscope standards are individually colored proteins for easy band identification***
- ***Prestained standards produce a consistent blue color in each band***



A. Kaleidoscope prestained standards. B. Prestained SDS-PAGE standards are a consistent blue color, and are available in High, Low, and Broad molecular weights.

Biotinylated Molecular Weight Standards

- ***Accurate calibration of molecular weights on a blot***
- ***Biotin does not alter the molecular weight of the individual protein***
- ***Use with colorimetric or chemiluminescent blot detection***



Biotinylated SDS-PAGE Standards, low and high range.

1. Avidin-HRP or Avidin-Ap is added to the secondary antibody solution.
2. Avidin binds to biotin, while the secondary antibody conjugate binds to the primary antibody.
3. Color development of the standards and the protein of interest is conducted simultaneously with common substrate.

Protein Molecular Weights

For more information on biotinylated protein molecular weights, see page 51.

Ordering Information

Catalog No.	Product Description
Protein Blotting Standards	
161-0324	Kaleidoscope Prestained Standards, 500 µl, for 100 mini blots
161-0325	Kaleidoscope Polypeptide Standards, 500 µl, for 100 mini blots
161-0305	Prestained SDS-PAGE Standards, low range, for 100 mini blots
161-0309	Prestained SDS-PAGE Standards, high range, for 100 mini blots
161-0318	Prestained SDS-PAGE Standards, broad range, for 100 mini blots
161-0306	Biotinylated SDS-PAGE Standards, low range, 250 µl
161-0311	Biotinylated SDS-PAGE Standards, high range, 250 µl
161-0319	Biotinylated SDS-PAGE Standards, broad range, 250 µl
170-6533	Avidin-AP, 1 ml
170-6528	Avidin-HRP, 2 ml
170-3554	Streptavidin-AP, 0.5 ml

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The On-Site Access Program is a new feature to the Bio-Rad line of reagents and consumables. This program keeps your lab stocked with all the reagents you need.

- ***Ready Gels***
- ***Standards***
- ***Buffers***
- ***Stains***
- ***Membranes***
- ***Reagents available 24 hours a day in your lab area.***

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