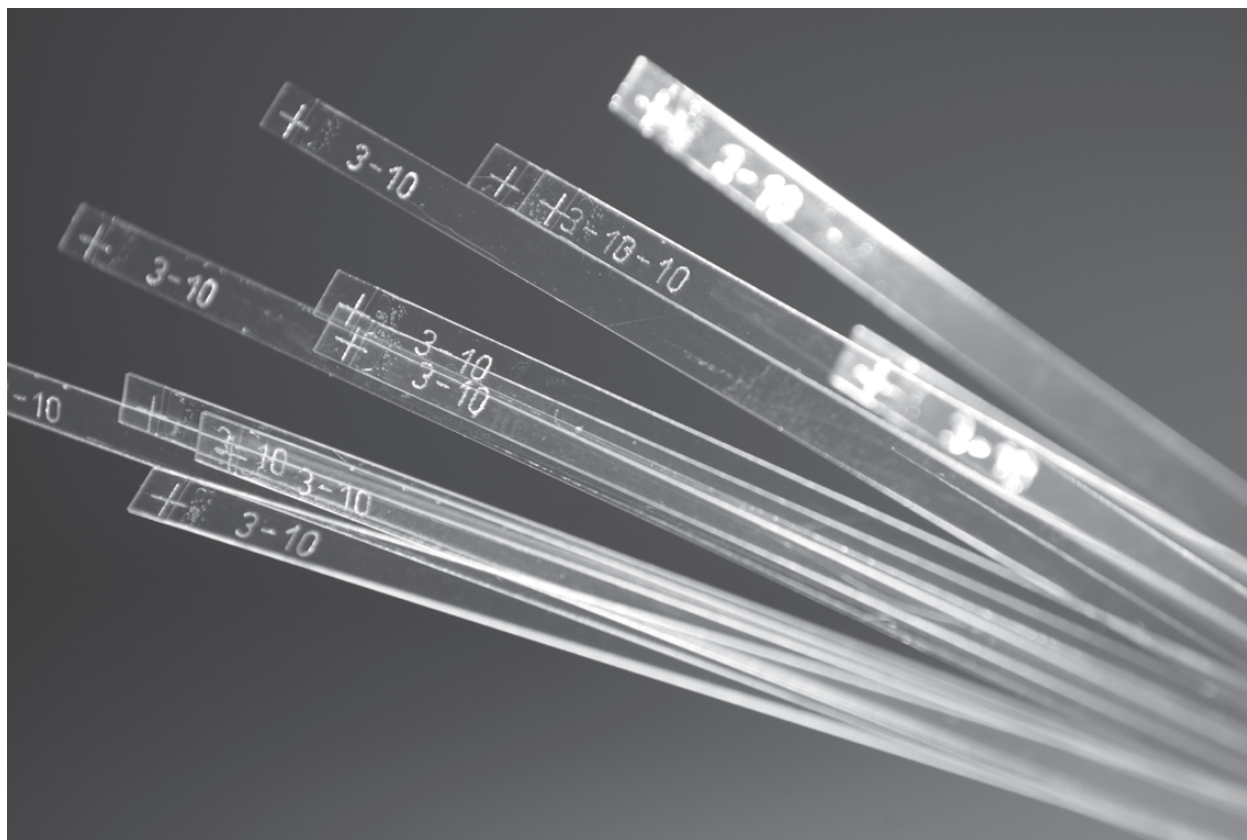


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# ReadyStrip IPG Strip

## Instruction Manual



**BIO-RAD**

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## Section 1 Introduction

The combination of isoelectric focusing (IEF) with SDS-PAGE provides a powerful technique for the separation of complex mixtures of proteins. ReadyStrip IPG Strips are made with buffering acrylamide derivatives that contain either a free carboxylic acid or a tertiary amino group, which is copolymerized with acrylamide and bis-acrylamide. As such, the pH gradient is precast into the gel and cannot shift during electrophoresis. The precast IPG strips provide reproducible gradients in an easy-to-use format. The ReadyPrep 2-D Starter Kit (catalog #1632105) is recommended for performing 2-D electrophoresis using IPG strips or for performing troubleshooting and method development. More detailed information on 2-D techniques and specific information about related products can be found in the 2-D Electrophoresis Workflow How-To Guide (bulletin 2651) as well as product-specific instruction manuals.

### Specifications.

Strip Length	7 cm	11 cm	17 cm	18 cm	24 cm
<b>IPG Strip Dimensions</b>					
IPG strip length, cm	7.9	11.8	17.8	19.0	24.7
Gel length, cm	7.3	11.0	17.1	18.0	23.4
Strip width, mm	3.3	3.3	3.3	3.3	3.3
<b>Linear pH Gradient Range</b>					
Broad range	3–10	3–10	3–10	3–10	3–10
	3–10 NL*	3–10 NL*	3–10 NL*	3–10 NL*	3–10 NL*
Narrow range	3–6, 4–7,	3–6, 4–7,	3–6, 4–7,	3–6, 4–7,	3–6, 4–7,
	5–8, 7–10	5–8, 7–10	5–8, 7–10	5–8, 7–10	5–8, 7–10

When rehydrated, IPG strips will be approximately 0.5 mm thick. The gel composition for each IPG strip is 4%T/3%C. The anode (acidic) end of each IPG strip is indicated with a "+" symbol. Storage temperature is –20°C. Each package contains 12 ReadyStrip IPG Strips.

\* NL, nonlinear gradient.

## Section 2 Solutions

### Rehydration buffer.

Standard Method	Optimization Guidelines
8 M urea	7–9.8 M urea, 0–2 M thiourea*
2% CHAPS	1–4% CHAPS*
50 mM dithiothreitol (DTT)	15–100 mM DTT*
0.2% Bio-Lyte Ampholytes or 1x ReadyStrip Buffer 0.1–0.4% (w/v) Bio-Lyte Ampholytes**	

\* The amounts of urea, CHAPS, and DTT and of Bio-Lyte Ampholytes required depend on the sample solubility. The amounts listed here serve as a general guideline. The optimal rehydration solution composition for each sample type is best determined empirically.

\*\* Use pH range recommended in chart below or optimize for sample.

### Recommended carrier ampholyte mixtures by ReadyStrip IPG Strip pH range.

IPG Strip	Buffer	IPG Strip	Buffer
3–10	Bio-Lyte 3/10 Ampholyte	3–6	Bio-Lyte 3/10 Ampholyte
3–10 NL	Bio-Lyte 3/10 Ampholyte	5–8	Bio-Lyte 3/10 Ampholyte
4–7	Bio-Lyte 3/10 Ampholyte	7–10	ReadyStrip 7–10 Buffer*

\* When 100x ReadyStrip Buffers are diluted to 1x, the final sample yields a concentration of 0.2% Bio-Lyte Ampholytes.

### Rehydration volumes.

IPG strip length, cm	7	11	17	18	24
Volume, $\mu$ l	125	200	300	315	450

Twelve hours is the recommended rehydration time.

**Recommended protein load ranges, µg.**

Staining Method	Silver stain	Coomassie G-250	Fluorescent stain
<b>IPG Strip Length</b>			
7 cm	5–20	50–100	5–100
11 cm	20–50	100–200	20–200
17 cm	50–80	200–400	50–400
18 cm	50–80	200–400	50–400
24 cm	80–150	400–800	80–800

## Section 3

# Focusing Conditions

**Suggested conditions for broad and narrow ranges.**

	Starting Voltage, V	Final Voltage, V	Volt-Hours, Vh	Ramp Rate	Temperature, °C
<b>ReadyStrip pH 3–10, 3–10 NL, 4–7, 5–8*</b>					
7 cm	0	4,000	8–15,000	Rapid	20
11 cm	0	8,000	20–35,000	Rapid	20
17 and 18 cm	0	10,000	40–60,000	Rapid	20
24 cm	0	10,000	60–80,000	Rapid	20
<b>ReadyStrip pH 3–6 Focusing Conditions**, ***</b>					
7 cm	0	4,000	8–10,000	Rapid	20
11 cm	0	8,000	15–20,000	Rapid	20
17 and 18 cm	0	10,000	30–40,000	Rapid	20
24 cm	0	10,000	40–55,000	Rapid	20
<b>ReadyStrip pH 7–10*, †</b>					
7 cm	0	4,000	8–16,000	Rapid	20
11 cm	0	8,000	20–30,000	Rapid	20
17 and 18 cm	0	10,000	40–50,000	Rapid	20
24 cm	0	10,000	60–70,000	Rapid	20

\* The final voltage for each pH range may not be reached, but the total volt-hours given above are sufficient to properly focus samples with final voltages as low as 3,000 V (7 cm), 5,000 V (11 cm), and 7,000 V (17, 18, and 24 cm). A lower final voltage will increase total run time.

\*\* The final voltage for this pH range may not be reached, but the total volt-hours given above are sufficient to properly focus samples with final voltages as low as 2,000 V (7 cm), 3,000 V (11 cm), and 6,000 V (17, 18, and 24 cm). A lower final voltage will increase total run time.

\*\*\* Enhanced resolution and separation of proteins may be achieved using cup loading with sample application at the cathode (–) end of the IPG strip.

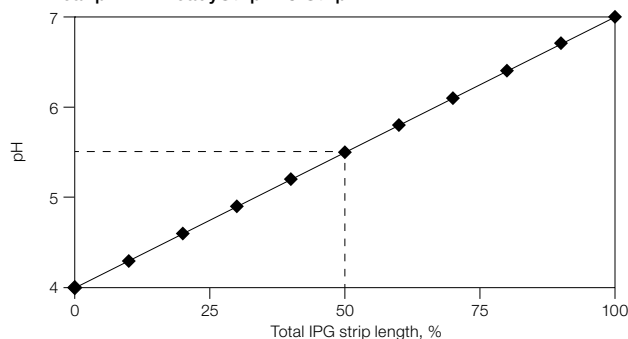
† To ensure success with basic range IPG strips, performing two additional steps is strongly recommended. The first step is to treat the sample using the ReadyPrep Reduction-Alkylation Kit (#1632090). This reduces streaking caused by disulfide bond formation, which is more problematic with basic range proteins. The second step is to use cup loading when loading samples for isoelectric focusing. For more information, refer to the ReadyPrep Reduction-Alkylation Kit Instruction Manual (bulletin 4110063).

## Section 4

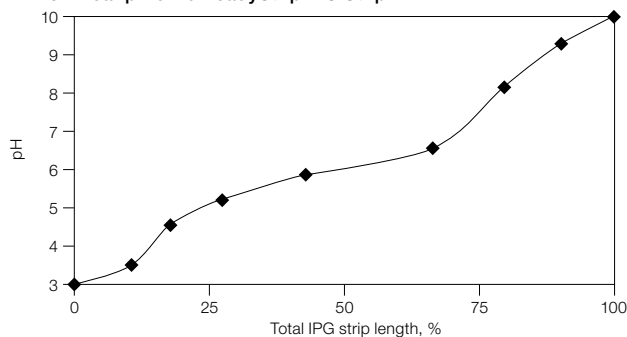
# Estimating the pI

By plotting the pH of a ReadyStrip IPG Strip as a function of its length, the pI of a protein may be derived from its focused position on that strip.

**A. Linear pH 4–7 ReadyStrip IPG Strip**



**B. Nonlinear pH 3–10 ReadyStrip IPG Strip**



**A**, the pH vs. length relationship of a linear pH 4–7 ReadyStrip IPG Strip, indicating how to estimate the pI of a protein that migrates to 50% of the gel length; **B**, the pH vs. length relationship of a nonlinear pH 3–10 ReadyStrip IPG Strip.

### Recommended equilibration volumes.

IPG Strip Length, cm	7	11	17	18	24
Equilibration buffer I, ml	2.5	4	6	6	8
Equilibration buffer II, ml	2.5	4	6	6	8

Ten minutes is recommended for each equilibration step.

### Second-dimension electrophoresis.

IPG Strip Length, cm	7	11	17	18	24
Electrophoresis cells	Mini-PROTEAN Tetra, PROTEAN Dodeca	Criterion, Criterion Dodeca	PROTEAN II XL	Use cell box that fits 20 x 20.5 cm gels	Use cell box that fits 25 x 20.5 cm gels
Electrophoresis conditions	200 V, constant	200 V, constant	16 mA/gel for 30 min, then 24 mA/gel for ~5 hr	200 V, constant	200 V, constant
Approximate run time	15–25 min	30–55 min	5.5 hr	6–8 hr	6–8 hr

## Section 5

# Barcoding

The 24 cm ReadyStrip IPG Strip is barcoded to assist in high-throughput applications. The automated barcode provides a unique 6-character identifier for each ReadyStrip IPG Strip. Within the barcoding area there is a human-readable portion for convenient information management without the use of a barcode reader. The figure below illustrates the human-readable and automated barcoding regions.



## Section 6

# Ordering Information

### ReadyStrip IPG Strips, 12 per package.

pH Range	7 cm	11 cm	17 cm	18 cm	24 cm
3–10	1632000	1632014	1632007	1632032	1632042
3–10 NL*	1632002	1632016	1632009	1632033	1632043
4–7	1632001	1632015	1632008	1632034	1632044
3–6	1632003	1632017	1632010	1632035	1632045
5–8	1632004	1632018	1632011	1632036	1632046
7–10	1632005	1632019	1632012	1632037	1632047

\* NL, nonlinear gradient.

#### Catalog # Description

##### Sample Preparation Kits

1632130	<b>ReadyPrep 2-D Cleanup Kit</b> , 50 preps
1632089	<b>ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear)</b> , 50 preps
1632088	<b>ReadyPrep Protein Extraction Kit (Membrane 1)</b> , 50 preps
1632087	<b>ReadyPrep Protein Extraction Kit (Signal)</b> , 50 preps
1632086	<b>ReadyPrep Protein Extraction Kit (Total Protein)</b> , 20 preps
1632085	<b>ReadyPrep Protein Extraction Kit (Soluble/Insoluble)</b> , 20 preps
1632084	<b>ReadyPrep Protein Extraction Kit (Membrane II)</b> , 10 preps
1632090	<b>ReadyPrep Reduction-Alkylation Kit</b> , 100 preps
1632100	<b>ReadyPrep Sequential Extraction Kit</b> , 5–15 preps

##### Buffers and Reagents

1632094	<b>Bio-Lyte 3/10 Ampholyte</b> , 1 ml
1632093	<b>ReadyStrip 7–10 Buffer</b> , 1 ml
1632105	<b>ReadyPrep 2-D Starter Kit</b> , 1 kit
1632106	<b>ReadyPrep 2-D Starter Kit Rehydration/Sample Buffer</b> , 10 ml
1632103	<b>ReadyPrep Sequential Extraction Kit Reagent 2</b> , 10 ml
1632104	<b>ReadyPrep Sequential Extraction Kit Reagent 3</b> , 10 ml
1632083	<b>ReadyPrep 2-D Rehydration/Sample Buffer 1</b> , 10 ml
1632107	<b>ReadyPrep 2-D Starter Kit Equilibration Buffer I</b> , with DTT, 10 ml
1632108	<b>ReadyPrep 2-D Starter Kit Equilibration Buffer II</b> , without DTT or iodoacetamide, 20 ml
1632129	<b>Mineral Oil</b> , 500 ml
1632111	<b>ReadyPrep Overlay Agarose</b> , 50 ml
1632092	<b>PROTEAN Plus Overlay Agarose</b> , 125 ml
1610731	<b>Urea</b> , 1 kg
1610716	<b>Tris</b> , 1 kg
1610611	<b>DTT</b> , 5 g
1632101	<b>Tributylphosphine (TBP)</b> , 200 mM, 0.6 ml
1632109	<b>Iodoacetamide</b> , 30 g
1610460	<b>CHAPS</b> , 1 g
1610732	<b>10x Tris/Glycine/SDS</b> , 1 L
1610772	<b>10x Tris/Glycine/SDS</b> , 5 L cube

#### Catalog # Description

##### PROTEAN i12 IEF System and Accessories

1646000	<b>PROTEAN i12 IEF System</b> , includes basic unit, electrode assemblies, focusing and rehydration/equilibration trays, forceps, electrode wicks, mineral oil, cleaning accessories, IPG strips and sample/rehydration buffer, USB flash drives, styluses
1646001	<b>PROTEAN i12 IEF Cell</b> , includes basic unit, electrode assemblies, and 3 styluses. Focusing trays and other accessories sold separately.
1646107	<b>i12 7 cm Focusing Tray</b> , pkg of 1, 7 cm focusing tray, holds up to twelve 7 cm IPG strips, includes 2 IPG strip retainers for gel-side down applications
1646111	<b>i12 11 cm Focusing Tray</b> , pkg of 1, 11 cm focusing tray, holds up to twelve 11 cm IPG strips, includes 2 IPG strip retainers for gel-side down applications
1646113	<b>i12 13 cm Focusing Tray</b> , pkg of 1, 13 cm focusing tray, holds up to twelve 13 cm IPG strips, includes 2 IPG strip retainers for gel-side down applications
1646117	<b>i12 17 cm Focusing Tray</b> , pkg of 1, 17 cm focusing tray, holds up to twelve 17 cm IPG strips, includes 2 IPG strip retainers for gel-side down applications
1646118	<b>i12 18 cm Focusing Tray</b> , pkg of 1, 18 cm focusing tray, holds up to twelve 18 cm IPG strips, includes 2 IPG strip retainers for gel-side down applications
1646124	<b>i12 24 cm Focusing Tray</b> , pkg of 1, 24 cm focusing tray, holds up to twelve 24 cm IPG strips, includes 2 IPG strip retainers for gel-side down applications
1654035	<b>i12 7 cm Rehydration/Equilibration Trays</b> , pkg of 25, 7 cm rehydration/equilibration trays, hold up to twelve 7 cm IPG strips
1654025	<b>i12 11 cm Rehydration/Equilibration Trays</b> , pkg of 25, 11 cm rehydration/equilibration trays, hold up to twelve 11 cm IPG strips
1646313	<b>i12 13 cm Rehydration/Equilibration Trays</b> , pkg of 25, 13 cm rehydration/equilibration trays, hold up to twelve 13 cm IPG strips
1654015	<b>i12 17 cm Rehydration/Equilibration Trays</b> , pkg of 25, 17 cm rehydration/equilibration trays, hold up to twelve 17 cm IPG strips
1654041	<b>i12 18 cm Rehydration/Equilibration Trays</b> , pkg of 25, 18 cm rehydration/equilibration trays, hold up to twelve 18 cm IPG strips
1654043	<b>i12 24 cm Rehydration/Equilibration Trays</b> , pkg of 25, 24 cm rehydration/equilibration trays, hold up to twelve 24 cm IPG strips
1646040	<b>IPG Strip Retainers</b> , pkg of 2, replacement IPG strip retainers for use with all sizes of PROTEAN i12 focusing trays
1646020	<b>i12 Sample Cup Holder</b> , pkg of 1, 12-position sample cup holder, includes 25 disposable sample cups (#1646021)
1646021	<b>i12 Sample Cups</b> , pkg of 25, disposable sample cups, for use with the PROTEAN i12 IEF system sample cup holder (#1646020)

Catalog #	Description
1646030	<b>Gel-Side Up Electrode Wicks</b> , pkg of 100, precut electrode wicks, for gel-side up applications
1646031	<b>Gel-Side Down Electrode Wicks</b> , pkg of 500, precut electrode wicks, for gel-side down applications
1646012	<b>Negative Electrode Assembly</b> , pkg of 1, replacement negative electrode assembly, for use with the PROTEAN i12 IEF system, can be used with all sizes of i12 focusing trays
1646011	<b>Positive Electrode Assembly</b> , pkg of 1, replacement positive electrode assembly, can be used with all sizes of i12 focusing trays
1646010	<b>Electrode Assembly Pair</b> , pkg of 1 pair, positive and negative electrode assemblies, can be used with all sizes of i12 focusing trays
1654072	<b>Cleaning Brushes</b> , pkg of 2
1610722	<b>Cleaning Concentrate</b> , 1 kg, 50x alkaline detergent
1646060	<b>USB Flash Drives</b> , pkg of 2, 2 GB flash drives, compatible with the PROTEAN i12 IEF system, for transferring data from the PROTEAN i12 IEF system to a computer for data analysis
1646050	<b>Styluses</b> , pkg of 3, for use on the PROTEAN i12 IEF system touch-screen user interface
1654070	<b>Forceps</b> , pkg of 1 pair, fine-tipped forceps for handling IPG strips
1655131	<b>AnyGel Stand</b> , pkg of 1, 6-row gel stand, holds 6 PROTEAN gels, 12 Criterion gels, or 18 Mini-PROTEAN gels
3459920	<b>Criterion Staining/Blotting Trays</b> , pkg of 12, gel staining trays, with lids

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