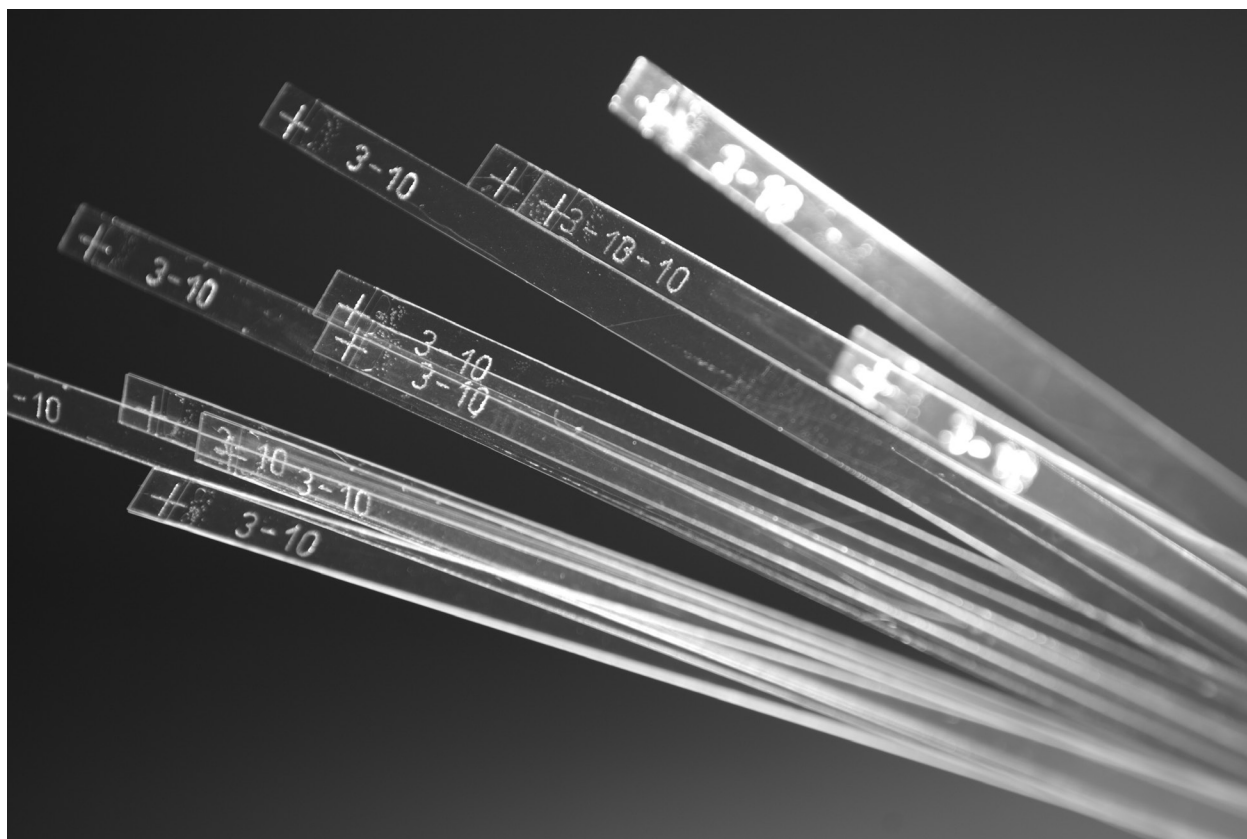


---

# ReadyStrip™ IPG Strip

## Instruction Manual



For technical service call your local Bio-Rad office or in the US call 1-800-4BIORAD (1-800-424-6723)



## 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 #163-2105) 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 Manual for Proteomics (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
Micro range	3.9–5.1,	3.9–5.1,	3.9–5.1,	3.9–5.1,	3.9–5.1,
	4.7–5.9,	4.7–5.9,	4.7–5.9,	4.7–5.9,	4.7–5.9,
	5.5–6.7,	5.5–6.7,	5.5–6.7,	5.5–6.7,	5.5–6.7,
	6.3–8.3	6.3–8.3	6.3–8.3	6.3–8.3	6.3–8.3

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 IPG strips.

\* NL, Nonlinear gradient.

## 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, DTT, and 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.

### Recommended carrier ampholyte mixtures by IPG strip pH range

IPG Strip	Buffer	IPG Strip	Buffer
3–10	Bio-Lyte 3/10	7–10	ReadyStrip 7–10 buffer*
3–10 NL	Bio-Lyte 3/10	3.9–5.1	ReadyStrip 3.9–5.1 buffer*
4–7	Bio-Lyte 3/10	4.7–5.9	ReadyStrip 4.7–5.9 buffer*
3–6	Bio-Lyte 3/10	5.5–6.7	ReadyStrip 5.5–6.7 buffer*
5–8	Bio-Lyte 3/10	6.3–8.3	ReadyStrip 6.3–8.3 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	7 cm	11 cm	17 cm	18 cm	24 cm
Volume	125 µl	200 µl	300 µl	315 µl	450 µl

12 hours is the recommended rehydration time.

### Recommended protein load ranges

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

## Focusing Conditions

### Broad and narrow ranges

	Start Voltage	Find Voltage	Volt-Hours	Ramp	Temperature
<b>ReadyStrip pH 3–10, 3–10 NL, 4–7, 5–8*</b>					
7 cm	0 V	4,000 V	8–15,000 V-hr	Rapid	20°C
11 cm	0 V	8,000 V	20–35,000 V-hr	Rapid	20°C
17 cm and					
18 cm	0 V	10,000 V	40–60,000 V-hr	Rapid	20°C
24 cm	0 V	10,000 V	60–80,000 V-hr	Rapid	20°C

### ReadyStrip pH 3–6 Focusing Conditions\*\*

7 cm	0 V	4,000 V	8–10,000 V-hr	Rapid	20°C
11 cm	0 V	8,000 V	15–20,000 V-hr	Rapid	20°C
17 cm and					
18 cm	0 V	10,000 V	30–40,000 V-hr	Rapid	20°C
24 cm	0 V	10,000 V	40–55,000 V-hr	Rapid	20°C

### ReadyStrip pH 7–10\* †

7 cm	0 V	4,000 V	8–16,000 V-hr	Rapid	20°C
11 cm	0 V	8,000 V	20–30,000 V-hr	Rapid	20°C
17 cm and					
18 cm	0 V	10,000 V	40–50,000 V-hr	Rapid	20°C
24 cm	0 V	10,000 V	60–70,000 V-hr	Rapid	20°C

### Micro ranges

	Start Voltage	End Voltage	Set Time	Ramp	Temperature
<b>ReadyStrip pH 3.9–5.1, and 4.7–5.9***</b>					

#### Step 1

7 cm	0 V	250 V	15 min	Rapid	20°C
11 cm	0 V	250 V	20 min	Rapid	20°C
17 cm and					
18 cm	0 V	250 V	30 min	Rapid	20°C
24 cm	0 V	250 V	30 min	Rapid	20°C

#### Step 2

7 cm	250 V	4,000 V	1 hr	Gradual	20°C
11 cm	250 V	8,000 V	1 hr	Gradual	20°C
17 cm and					
18 cm	250 V	10,000 V	2 hr	Gradual	20°C
24 cm	250 V	10,000 V	2 hr	Gradual	20°C

#### Step 3

7 cm	4,000 V	4,000 V	10–20,000 V-hr	Rapid	20°C
11 cm	8,000 V	8,000 V	20–32,000 V-hr	Rapid	20°C
17 cm and					
18 cm	10,000 V	10,000 V	30–50,000 V-hr	Rapid	20°C
24 cm	10,000 V	10,000 V	60–90,000 V-hr	Rapid	20°C

### ReadyStrip pH 5.5–6.7, and 6.3–8.3†

#### Step 1

7 cm	0 V	250 V	15 min	Rapid	20°C
11 cm	0 V	250 V	20 min	Rapid	20°C
17 cm and					
18 cm	0 V	250 V	30 min	Rapid	20°C
24 cm	0 V	250 V	30 min	Rapid	20°C

#### Step 2

7 cm	250 V	4,000 V	1 hr	Gradual	20°C
11 cm	250 V	8,000 V	1 hr	Gradual	20°C
17 cm and					
18 cm	250 V	10,000 V	2 hr	Gradual	20°C
24 cm	250 V	10,000 V	2 hr	Gradual	20°C

#### Step 3

7 cm	4,000 V	4,000 V	20–30,000 V-hr	Rapid	20°C
11 cm	8,000 V	8,000 V	30–45,000 V-hr	Rapid	20°C
17 cm and					
18 cm	10,000 V	10,000 V	45–63,000 V-hr	Rapid	20°C
24 cm	10,000 V	10,000 V	60–90,000 V-hr	Rapid	20°C

\* 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 cm, 18 cm, and 24 cm). A lower final voltage will increase total run time.

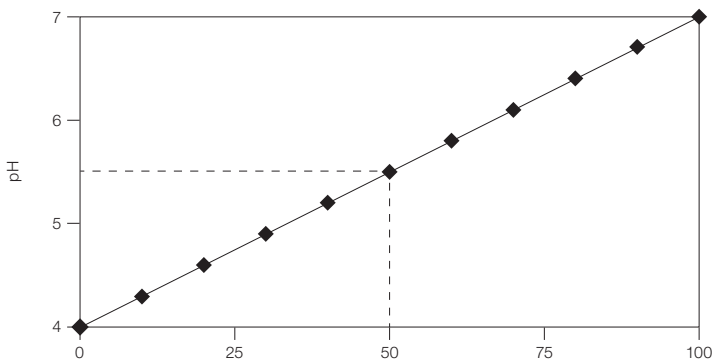
\*\* The final voltage for this pH range may not be reached, but the total volt-hours given above are sufficient to properly focus samples with final voltages as low as 2,000 V (7 cm), 3,000 V (11 cm), and 6,000 V (17 cm, 18 cm, and 24 cm). A lower final voltage will increase total run time.

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

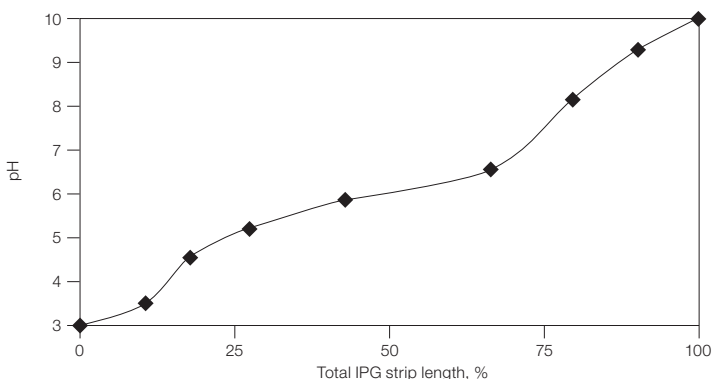
† To ensure success with basic range IPG strips, performing two additional steps is strongly recommended. The first step is to treat the sample using the ReadyPrep reduction-alkylation kit (catalog #163-2090). This reduces streaking caused by disulfide bond formation, which is more problematic with basic 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).

## Estimating the pI of a protein from its position along an IPG strip

### A. Linear pH 4–7 ReadyStrip IPG strip



### B. Nonlinear pH 3–10 ReadyStrip IPG strip



By plotting the pH of an IPG strip as a function of its length, the pI of a protein may be derived from its focused position on that strip. **A**, the pH vs. length relationship of a linear pH 4–7 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 IPG strip.

## Recommended equilibration volumes

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

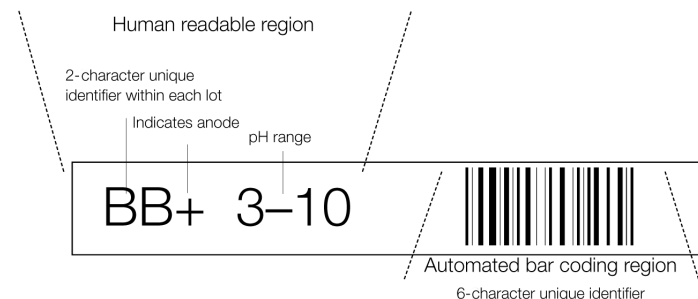
10 minutes is recommended for each equilibration step.

## Second dimension electrophoresis

IPG Strip Length	7 cm	11 cm	17 cm	18 cm	24 cm
Electrophoresis cells	Mini-PROTEAN® Tetra, PROTEAN® and Dodeca	Criterion™ Criterion Dodeca	PROTEAN II XL	PROTEAN Plus Dodeca	PROTEAN Plus Dodeca
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

## Bar Coding

The 24 cm IPG strip contains bar coding to assist in high-throughput applications. The automated bar code provides a unique 6-character identifier for each IPG strip. The recommended bar code reader is the Gryphon L GD 4330 laser scanner (Part # GD4330-BKK1).<sup>\*</sup> Within the bar coding area there is a human readable portion for convenient information management without the use of a bar code reader. The figure below illustrates the human-readable and automated bar coding regions.



<sup>\*</sup> Infinite Corporate Solutions, Inc. 1400 Battleground Ave., Suite 207-B Greensboro, NC 27408, USA. Tel. 866-271-6556.

## Part Numbers

### ReadyStrip IPG strips, 12 per package

pH Range	7 cm	11 cm	17 cm	18 cm	24 cm
3–10	163-2000	163-2014	163-2007	163-2032	163-2042
3–10 NL*	163-2002	163-2016	163-2009	163-2033	163-2043
4–7	163-2001	163-2015	163-2008	163-2034	163-2044
3–6	163-2003	163-2017	163-2010	163-2035	163-2045
5–8	163-2004	163-2018	163-2011	163-2036	163-2046
7–10	163-2005	163-2019	163-2012	163-2037	163-2047
3.9–5.1	163-2028	163-2024	163-2020	163-2038	163-2048
4.7–5.9	163-2029	163-2025	163-2021	163-2039	163-2049
5.5–6.7	163-2030	163-2026	163-2022	163-2040	163-2050
6.3–8.3	163-2031	163-2027	163-2023	163-2041	163-2051

\* NL, Nonlinear gradient.

## Ordering Information

Catalog #	Description	Catalog #	Description
<b>Sample Preparation Kits</b>			
163-2130	ReadyPrep 2-D Cleanup Kit, 50 preps	165-4025	<b>i12 11 cm Rehydration/Equilibration Trays</b> , pkg of 25, 11 cm rehydration/equilibration trays, hold up to twelve 11 cm IPG strips, for use with the PROTEAN i12 IEF and the PROTEAN IEF systems
163-2089	ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear), 50 preps	164-6313	<b>i12 13 cm Rehydration/Equilibration Trays</b> , pkg of 25, 13 cm rehydration/equilibration trays, hold up to twelve 13 cm IPG strips, for use with the PROTEAN i12 IEF and the PROTEAN IEF systems
163-2088	ReadyPrep Protein Extraction Kit (Membrane 1), 50 preps	165-4015	<b>i12 17 cm Rehydration/Equilibration Trays</b> , pkg of 25, 17 cm rehydration/equilibration trays, hold up to twelve 17 cm IPG strips, for use with the PROTEAN i12 IEF and the PROTEAN IEF systems
163-2087	ReadyPrep Protein Extraction Kit (Signal), 50 preps	165-4041	<b>i12 18 cm Rehydration/Equilibration Trays</b> , pkg of 25, 18 cm rehydration/equilibration trays, hold up to twelve 18 cm IPG strips, for use with the PROTEAN i12 IEF and the PROTEAN IEF systems
163-2086	ReadyPrep Protein Extraction Kit (Total Protein), 20 preps	165-4043	<b>i12 24 cm Rehydration/Equilibration Trays</b> , pkg of 25, 24 cm rehydration/equilibration trays, hold up to twelve 24 cm IPG strips, for use with the PROTEAN i12 IEF and the PROTEAN IEF systems
163-2085	ReadyPrep Protein Extraction Kit (Soluble/Insoluble), 20 preps	164-6040	<b>IPG Strip Retainers</b> , pkg of 2, replacement IPG strip retainers for use with all sizes of i12 focusing trays
163-2084	ReadyPrep Protein Extraction Kit (Membrane II), 10 preps	164-6020	<b>i12 Sample Cup Holder</b> , pkg of 1, 12-position sample cup holder, includes 25 disposable sample cups (#164-6021), for use with the PROTEAN i12 IEF system
163-2090	ReadyPrep Reduction-Alkylation Kit, 100 preps	164-6021	<b>i12 Sample Cups</b> , pkg of 25, disposable sample cups, for use with the PROTEAN i12 IEF system sample cup holder (#164-6020)
163-2100	ReadyPrep Sequential Extraction Kit, 5–15 preps	164-6030	<b>Gel-Side Up Electrode Wicks</b> , pkg of 100, precut electrode wicks, for use with the PROTEAN i12 IEF system for gel-side up applications
<b>Buffers and Reagents</b>			
163-2094	Bio-Lyte 3/10 Ampholyte, 1 ml	164-6031	<b>Gel-Side Down Electrode Wicks</b> , pkg of 500, precut electrode wicks, for use with the PROTEAN i12 IEF system for gel-side down applications
163-2093	ReadyStrip 7–10 Buffer, 1 ml	164-6012	<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
163-2098	ReadyStrip 3.9–5.1 Buffer, 1 ml	164-6011	<b>Positive Electrode Assembly</b> , pkg of 1, replacement positive electrode assembly, for use with the PROTEAN i12 IEF system, can be used with all sizes of i12 focusing trays
163-2097	ReadyStrip 4.7–5.9 Buffer, 1 ml	164-6010	<b>Electrode Assembly Pair</b> , pkg of 1 pair, positive and negative electrode assemblies, for use with the PROTEAN i12 IEF system, can be used with all sizes of i12 focusing trays
163-2096	ReadyStrip 5.5–6.7 Buffer, 1 ml	165-4072	<b>Cleaning Brushes</b> , pkg of 2, cleaning brushes, for cleaning the focusing and rehydration/equilibration trays used with the PROTEAN i12 IEF and PROTEAN IEF systems
163-2095	ReadyStrip 6.3–8.3 Buffer, 1 ml	161-0722	<b>Cleaning Concentrate</b> , 1 kg, concentrated cleaning solution for use with the PROTEAN i12 IEF and PROTEAN IEF systems
163-2105	ReadyPrep 2-D Starter Kit, 1 kit	164-6060	<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
163-2106	ReadyPrep 2-D Starter Kit Rehydration/Sample Buffer, 10 ml	164-6050	<b>Styluses</b> , pkg of 3, for use on the PROTEAN i12 IEF system touch-screen user interface
163-2103	ReadyPrep Sequential Extraction Kit Reagent 2, 10 ml	163-2129	<b>Mineral Oil</b> , 500 ml, for use with the PROTEAN i12 IEF and PROTEAN IEF systems
163-2104	ReadyPrep Sequential Extraction Kit Reagent 3, 10 ml	165-4070	<b>Forceps</b> , pkg of 1 pair, fine-tipped forceps for handling immobilized pH gradient (IPG) strips
163-2083	ReadyPrep 2-D Rehydration/Sample Buffer 1, 10 ml	165-5131	<b>AnyGel™ Stand</b> , pkg of 1, 6-row gel stand, holds 6 PROTEAN gels, 12 Criterion gels, or 18 Ready Gel® mini gels
163-2107	ReadyPrep 2-D Starter Kit Equilibration Buffer I, with DTT, 10 ml	345-9920	<b>Criterion Staining/Blotting Trays</b> , pkg of 12, gel staining trays, with lids
163-2108	ReadyPrep 2-D Starter Kit Equilibration Buffer II, without DTT or iodoacetamide, 20 ml	<b>PROTEAN IEF Accessories</b>	
163-2129	Mineral Oil, 500 ml	165-4030	7 cm Focusing Tray, with lid, pkg of 1
163-2111	ReadyPrep Overlay Agarose, 50 ml	165-4020	11 cm Focusing Tray, with lid, pkg of 1
163-2092	PROTEAN Plus Overlay Agarose, 125 ml	165-4010	17 cm Focusing Tray, with lid, pkg of 1
161-0731	Urea, 1 kg	165-4040	18 cm Focusing Tray, with lid, pkg of 1
161-0716	Tris, 1 kg	165-4042	24 cm Focusing Tray, with lid, pkg of 1
161-0611	DTT, 5 g	165-4050	Cup Loading Tray, pkg of 1
163-2101	Tributylphosphine (TBP), 200 mM, 0.6 ml	165-4051	Large Replacement Cups, 150 µl, pkg of 120
163-2109	Iodoacetamide, 30 g	165-4052	Small Replacement Cups, 100 µl, pkg of 120
161-0460	CHAPS, 1 g	165-4053	Replacement Movable Electrodes, 1 pair
161-0732	10x Tris/Glycine/SDS, 1 L	165-4054	Replacement Cup Loading Tray Base
161-0772	10x Tris/Glycine/SDS, 5 L cube	Coomassie is a trademark of BASF Aktiengesellschaft. Gryphon is a trademark of Datalogic Scanning, Inc.	
<b>PROTEAN® i12™ IEF Cell and Accessories</b>			
164-6000	PROTEAN i12 IEF Isoelectric Focusing System, 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	164-6060	
164-6001	PROTEAN i12 IEF Isoelectric Focusing Cell, includes basic unit, electrode assemblies, and 3 styluses. Focusing trays and other accessories sold separately.	164-6050	
164-6107	<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, for use with the PROTEAN i12 IEF system	163-2129	
164-6111	<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, for use with the PROTEAN i12 IEF system	165-5131	
164-6113	<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, for use with the PROTEAN i12 IEF system	345-9920	
164-6117	<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, for use with the PROTEAN i12 IEF system	<b>PROTEAN IEF Accessories</b>	
164-6118	<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, for use with the PROTEAN i12 IEF system	165-4030	7 cm Focusing Tray, with lid, pkg of 1
164-6124	<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, for use with the PROTEAN i12 IEF system	165-4020	11 cm Focusing Tray, with lid, pkg of 1
165-4035	<b>i12 7 cm Rehydration/Equilibration Trays</b> , pkg of 25, 7 cm rehydration/equilibration trays, hold up to twelve 7 cm IPG strips, for use with the PROTEAN i12 IEF and the PROTEAN IEF systems	165-4010	17 cm Focusing Tray, with lid, pkg of 1



**BIO-RAD**

**Bio-Rad  
Laboratories, Inc.**

Life Science  
Group

Web site [www.bio-rad.com](http://www.bio-rad.com) USA 800 424 6723 Australia 61 2 9914 2800 Austria 01 877 89 01 Belgium 09 385 55 11 Brazil 55 31 3689 6600  
Canada 905 364 3435 China 86 21 6169 8500 Czech Republic 420 241 430 532 Denmark 44 52 10 00 Finland 09 804 22 00  
France 01 47 95 69 65 Germany 089 31 884 0 Greece 30 210 777 4396 Hong Kong 852 2789 3300 Hungary 36 1 459 6100 India 91 124 4029300  
Israel 03 963 6050 Italy 39 02 216091 Japan 03 6361 7000 Korea 82 2 3473 4460 Malaysia 60 3 2117 5260 Mexico 52 555 488 7670  
The Netherlands 0318 540666 New Zealand 64 9 415 2280 Norway 23 38 41 30 Poland 48 22 331 99 99 Portugal 351 21 472 7700  
Russia 7 495 721 14 04 Singapore 65 6415 3170 South Africa 27 861 246 723 Spain 34 91 590 5200 Sweden 08 555 12700  
Switzerland 061 717 95 55 Taiwan 886 2 2578 7189 Thailand 66 2 6518311 United Kingdom 020 8328 2000