ReadyStrip™ IPG Strip Instruction Manual

Catalog #
163-2099

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BIO-RAD

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Section 1
Introduction

The combination of IEF with SDS-PAGE provides a powerful technique for the separation of complex mixtures of proteins. IEF separations performed in tube gels using ampholytes to form the pH gradient during electrophoresis can exhibit performance problems such as gradient drift and batch variability. The precast immobilized pH gradient IPG gel strip eliminates these problems. IPG strips are made with buffering acrylamide derivatives that contain either a free carboxylic acid or a tertiary amino group that 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 provides reproducible gradients in an easy-to-use format.

The procedures described in this manual can be performed using the Bio-Rad ReadyPrep™ 2-D starter kit (catalog #163-2105) to learn how to perform 2-D electrophoresis using IPG strips, or to perform troubleshooting and method development.

Section 2
Specifications

	Strip Length				
	7 cm	**11 cm**	**17 cm**	**18 cm**	**24 cm**
Strip dimensions					
Strip length	7.9 cm	11.8 cm	17.8 cm	19.0 cm	24.7 cm
Gel length	7.25 cm	11.0 cm	17.1 cm	18.0 cm	23.4 cm
Strip width	3.3 mm	3.3 mm	3.3 mm	3.3 mm	3.3 mm
Gel thickness	0.5 mm	0.5 mm	0.5 mm	0.5 mm	0.5 mm
Linear pH gradient range					
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
Gel composition	4%T/3%C	4%T/3%C	4%T/3%C	4%T/3%C	4%T/3%C
Storage	-20°C	-20°C	-20°C	-20°C	-20°C
Number of strips/package	12	12	12	12	12
Anode (acidic) end identified by:	+	+	+	+	+

*Nonlinear gradient

Section 3
Rehydration and Sample Application

Note: Always wear laboratory gloves when handling IPG strips and all apparatus or solutions used in their preparation to prevent protein contamination, primarily from skin keratin.

Rehydrate and load IPG strips using one of the three basic methods given below. **Method 1, rehydration in the rehydration/equilibration tray,** is a convenient method that prevents cross-contamination of protein samples by utilizing a disposable tray for rehydration. Sample is included in the rehydration solution and the sample is taken up into the IPG strip passively during rehydration (passive rehydration). **Method 2, rehydration in the PROTEAN® IEF focusing tray,** allows rehydration under current (active rehydration), which can improve entry of high molecular weight proteins into the IPG strip[1]. Sample is included in the rehydration solution in this method as well. This method also allows the possibility of having IEF commence automatically following rehydration without user intervention. **Method 3, cup loading,** is a method in which the sample is applied via sample cups following rehydration, rather than being included in the rehydration solution. This method is less convenient, but can result in better focusing, particularly when the pH range of the IPG strip is alkaline.

3.1 Method 1, Rehydration in the Rehydration/Equilibration Tray

1. Prepare sample in, or dilute into, a suitable rehydration buffer. The rehydration buffer should contain urea, a nonionic or zwitterionic detergent, carrier ampholytes, and a reducing agent. The optimal buffer composition and protein load will be sample-dependent. See section 6 for recommended buffers and for more information on sample and rehydration buffer composition.

2. Using the table below, pipet the indicated volume of each sample as a line along the edge of a channel in a new or clean, dry disposable rehydration/equilibration tray. The line of sample should extend along the whole length of the channel except for about 1 cm at each end. Take care not to introduce any bubbles, which may interfere with the even distribution of sample in the IPG strip (see Figure 1).

	7 cm	**11 cm**	**17 cm**	**18 cm**	**24 cm**
Rehydration volume	125 µl	200 µl	300 µl	315 µl	450 µl

3. When all the protein samples have been loaded into the rehydration/equilibration tray as pictured in Figure 1, peel the cover sheet from the IPG strip using forceps, as shown in Figure 2.

4. Gently place the IPG strip(s), gel side down, onto the sample. The "+" and the pH range marked on the IPG strip should be legible. Take care not to get the sample onto the plastic backing of the IPG strips as this portion of the sample will not be absorbed by the gel material. Also take care not to trap air bubbles beneath the IPG strip. If this happens, carefully use the forceps to lift the IPG strip up and down from one end until the air bubbles move to the end and out from under the IPG strip.

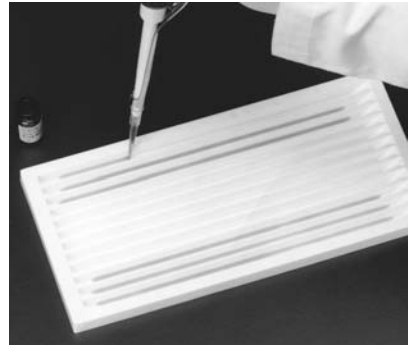


Fig. 1. Sample loading in rehydration/equilibration trays.

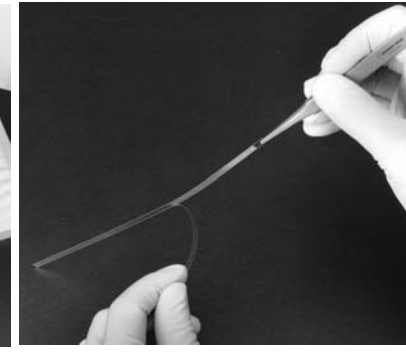


Fig. 2. Removing the cover sheet from the IPG strip.

5. Overlay each of the IPG strips with 2–3 ml of mineral oil (catalog #163-2129) to prevent evaporation during the rehydration process. Add the mineral oil slowly by carefully dripping the oil onto the plastic backing of the IPG strip while moving the pipet along the length of the IPG strip. Alternatively, wait approximately 1 hr after starting rehydration, until most of the liquid has been absorbed by the IPG strip. Then overlay the IPG strip with mineral oil.
6. Cover the rehydration/equilibration tray with the plastic lid provided and leave the tray sitting on a level bench overnight (11–16 hr) to rehydrate the IPG strips and load the protein sample.
7. Place a clean, dry PROTEAN® IEF focusing tray onto the lab bench.
8. Using forceps, place a paper wick (catalog #165-4071) at each end of the channels so that the wire electrode is covered.
9. Pipet 10 µl of deionized (18 MΩ-cm) water onto each wick to wet them. Readjust their position if necessary.
10. Remove the cover from the rehydration/equilibration tray containing the IPG strips. Using forceps, carefully hold the strip vertically for about 7–8 sec and blot the tip of the strip on a piece of filter paper to allow the mineral oil to drain (Figure 3), then transfer the IPG strip to the corresponding channel in the

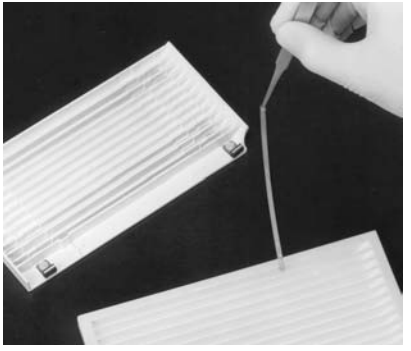


Fig. 3. Draining the oil.

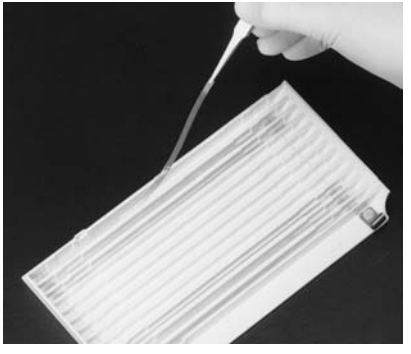


Fig. 4. Placing the IPG strip gel side down in the focusing tray.

focusing tray (maintain the gel side down) (Figure 4). Remember to observe the correct polarity during the transfer. The "+" marked on the IPG strip should be positioned at the end of the tray marked "+".

Draining the oil allows the removal of unabsorbed protein from the surface of the gel and reduces the incidence of horizontal streaking. Alternatively, the oil can be removed by gentle blotting. Place the IPG strips gel side up on a piece of dry filter paper. Wet a second piece of filter paper and place it gently onto the IPG strips. Carefully pat the paper above the IPG strips to remove the oil. Finally, gently peel back the top layer of filter paper, starting from one end.

11. Cover each IPG strip with 2–3 ml of fresh mineral oil. Check for any air bubbles trapped beneath the IPG strips and if necessary, remove them by lifting the IPG strip from one end and carefully placing it back in the channel. Place the lid onto the focusing tray.
12. Place the focusing tray into the PROTEAN IEF cell and close the cover.

    Program the PROTEAN IEF cell using the appropriate protocol in section 4. For all IPG strip lengths, use the default cell temperature of 20ºC, with a maximum current of 50 µA/IPG strip. Refer to the PROTEAN IEF Cell Instruction Manual for details on entering run parameters.

    Press START to initiate the electrophoresis run.
13. When the electrophoresis run has been completed, remove the IPG strips from the focusing tray and transfer them gel side up into a new clean, dry disposable rehydration/equilibration tray which matches the length of the IPG strip. Hold the IPG strips vertically with forceps and let the mineral oil drain from the IPG strip for ~5 sec before transfer. Maintain the IPG strips in the same order as in the focusing tray.
14. If you are not proceeding directly to the equilibration step (see Section 5), cover the tray containing the IPG strips, wrap it in plastic wrap, and place in a –70ºC freezer for storage.

3.2 Method 2: Rehydration in the PROTEAN® IEF Focusing Tray

1. Prepare sample in, or dilute into, a suitable rehydration buffer. The rehydration buffer should contain urea, a nonionic or zwitterionic detergent, carrier ampholytes, and a reducing agent. The optimal buffer composition and protein load will be sample-dependent. See section 6 for recommended buffers and for more information on sample and rehydration buffer composition.
2. Using the table below, pipet the indicated volume of each sample as a line along the edge of a channel in an IEF focusing tray. The line of sample should extend along the whole length of the channel except for about 1 cm at each end. Take care not to introduce any bubbles, which may interfere with the even distribution of sample in the IPG strip.

	7 cm	11 cm	17 cm	18 cm	24 cm
Rehydration volume	125 µl	200 µl	300 µl	315 µl	450 µl

3. Peel the cover sheet from the IPG strip using forceps, as shown in Figure 2. Place the IPG strip(s) gel side down in the IEF focusing tray (Figure 4) so that the acidic (marked with "+") end is at the anode (red/+) of the IEF cell. Ensure that the gels make contact with the electrodes. Take care not to get the sample onto the plastic backing of the IPG strip(s) as this portion of the sample will not be absorbed by the gel material. Also take care not to trap air bubbles beneath the IPG strip. If this happens, carefully use the forceps to lift the IPG strip up and down from one end until the air bubbles move to the end and out from under the IPG strip. Overlay with mineral oil.
4. Place the IEF focusing tray in the PROTEAN IEF cell. Rehydrate under active or passive conditions for at least 12 hr. Refer to the PROTEAN IEF Cell Instruction Manual for details of how to program and run the instrument.

   Note: The next three steps may be omitted if the IEF run has been set up for unattended operation from

rehydration through focusing. Best results are obtained when paper wicks are placed between the ends of the IPG strips and the wire electrodes following rehydration, but these steps are not always necessary and may often be omitted for the sake of convenience.

5. After rehydration is complete, wet two paper wicks (catalog #165-4071) with deionized water (10 µl per paper wick).
6. Carefully lift the ends of each IPG strip with forceps and insert a wet paper wick between the IPG strip and the electrodes (Figure 5).
7. Program the PROTEAN IEF cell using the appropriate protocol in section 4. For all IPG strip lengths, use the default cell temperature of 20ºC, with a maximum current of 50 µA/IPG strip. Refer to the PROTEAN IEF Cell Instruction Manual for details on entering run parameters.

   Press START to initiate the electrophoresis run.
8. When the electrophoresis run has been completed, remove the IPG strips from the focusing tray and transfer them gel side up into a new clean, dry disposable rehydration/equilibration tray which matches the length of the IPG strip. Hold the IPG strips vertically with forceps and let the mineral oil drain from the IPG strip for ~5 sec before transfer. Maintain the IPG strips in the same order as in the focusing tray.
9. If you are not proceeding directly to the equilibration step (see Section 5), cover the tray containing the IPG strips, wrap it in plastic wrap, and place in a –70ºC freezer for storage.

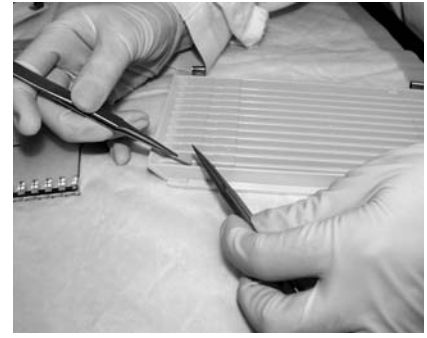


Fig. 5. Inserting electrode wicks between the IPG strip and electrode.

3.3 Method 3: Cup Loading

1. Prepare rehydration buffer and rehydrate IPG strips just prior to sample cup loading and isoelectric focusing. Add the indicated volume of rehydration buffer to each of the required channels in the rehydration/equilibration tray to rehydrate IPG strips to 0.53 mm thickness. Rehydration to this thickness ensures a complete seal between the cup and the IPG strip. Refer to the following table:

	7 cm	11 cm	17 cm	18 cm	24 cm
Rehydration volume for cup loading	135 µl	215 µl	330 µl	345 µl	460 µl

   Note: When cup loading, sample is not included in the rehydration solution.

2. Using the table above, pipet the indicated volume of rehydration solution as a line along the edge of a channel in a new or clean, dry disposable rehydration/equilibration tray. The line of rehydration buffer should extend along the whole length of the channel except for about 1 cm at each end. Take care not to introduce any bubbles, which may interfere with the even distribution of rehydration buffer in the IPG strip (see Figure 1)
3. When rehydration solution has been pipeted into the rehydration/equilibration tray as pictured in Figure 1, peel the cover sheet from the IPG strip using forceps, as shown in Figure 2.
4. Gently place the IPG strip(s), gel side down, onto the rehydration solution. The "+" and the pH range marked on the IPG strip should be legible. Take care not to get rehydration solution onto the plastic backing of the IPG strips as this portion of the solution will not be absorbed by the gel material. Also take care not to trap air bubbles beneath the IPG strip. If this happens, carefully use forceps to lift the IPG strip up and down from one end until the air bubble move to the end and out from under the IPG strip.
5. Overlay each of the IPG strips with 2–3 ml of mineral oil (catalog #163-2129) to prevent evaporation during the rehydration process. Add the mineral oil slowly by carefully dripping the oil onto the plastic

backing of the IPG strip while moving the pipet along the length of the IPG strip. Alternatively, wait approximately 1 hr after starting rehydration, until most of the liquid has been absorbed by the IPG strip. Then overlay the IPG strip with mineral oil.

6. Cover the rehydration/equilibration tray with the plastic lid provided and leave the tray sitting on a level bench overnight (11–16 hr) to rehydrate the IPG strips.
7. Remove the rehydrated IPG strips from the rehydration/equilibration tray. Remove excess mineral oil by carefully blotting the IPG strip on filter paper wetted with deionized water.
8. Place the rehydrated IPG strip, gel side up, in the cup loading tray (catalog #165-4050). Place the anode side (marked with "+" on IPG strips) of the 17 cm, 18 cm, and 24 cm IPG strips flush against the anode (left) side of the cup loading tray. Place the anode side of the 7 cm and 11 cm IPG strips approximately 4 cm from the anode side of the tray to ensure proper placement and contact of the cathode assembly with the electrode contact area (Figure 6).
9. Prepare two paper wicks (catalog #165-4071) for each IPG strip used. Using scissors, cut each paper wick to a width of 4.2 mm in order to fit into the cup loading tray. Wet each paper wick with 10 µl deionized water. Carefully place the damp paper wicks onto the gel surface at the ends of the IPG strip prior to positioning the anode and cathode electrode assemblies.
10. Place movable electrode assemblies at the cathode and anode ends of the IPG strips over the paper wicks. Make sure that the electrodes make contact with the electrode contact areas on the front of the cup loading tray.

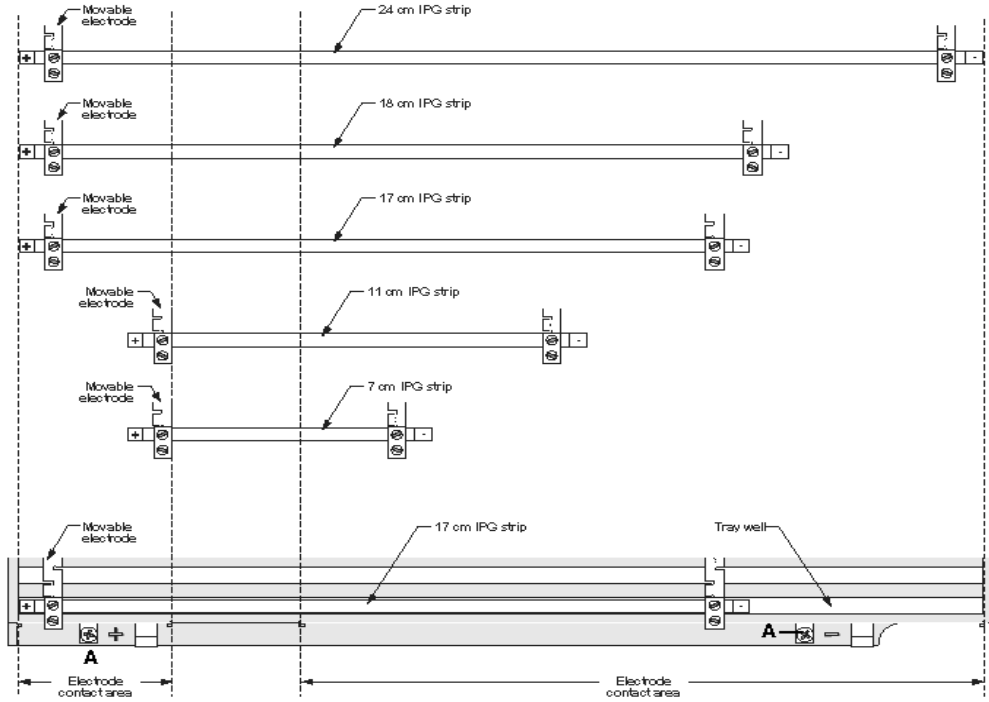


Fig 6. Correct placement regions for various IPG strip lengths.

Note: If the electrode screws, indicated by the letter “A” in Figure 6, on the front of the cup loading tray interfere with the placement of the moveable electrode assembly, reposition the IPG strips until the electrode assembly clears the electrode screws.

11. Determine which sample cup size will be used. The small sample cups (catalog #165-4051) are used for sample volumes up to 100 µl and the large sample cups (catalog #165-4052) are used for sample volumes up to 150 µl. Cut the required number of sample cups from a twelve-cup strip with scissors (Figure 7). A minimum of two sample cups is needed for a tight, leakproof fit in the cup loading tray.

    Note: When cutting the indent of the bridge between two cups, ensure that no burrs remain in the cut that would prevent the bridge from sitting directly on the wall that divides two channels in the cup loading tray.

12. Place the sample cups on the gel surface. In general, the sample cups should be placed near the

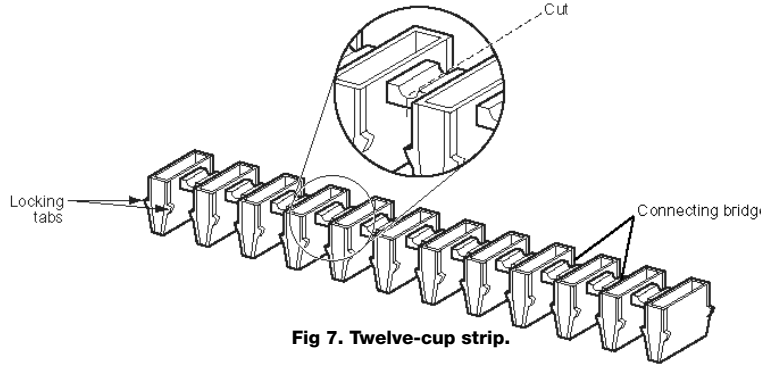


Fig 7. Twelve-cup strip.

anode (+) for basic pH range IPG strips and near the cathode (-) for acidic pH range IPG strips. There are, however, exceptions. Sample cup placement can influence resolution at the ends of the IPG strip, with resolution often best at the opposite end of the IPG strip from where the sample cup is placed. This can be exploited to improve resolution at whichever end of the pH range is of most interest. If the sample contains an anionic detergent such as SDS, the sample cup should be placed near the anode regardless of the pH range of the IPG strip. To position the sample cups properly, make sure that the bridges connecting the sample cups are pushed down onto each wall that divides the cup loading tray channels.

Note: Improper placement of the sample cup can result in sample leaking.

13. When the cups are securely positioned, add the protein sample to the sample cups (Figure 8). A maximum volume of 100 µl is recommended for the small sample cups and a maximum of 150 µl for the large sample cups. Improved resolution has been observed with smaller volumes containing more concentrated protein, and it is recommended to use the maximum protein concentration possible in which the proteins are soluble.

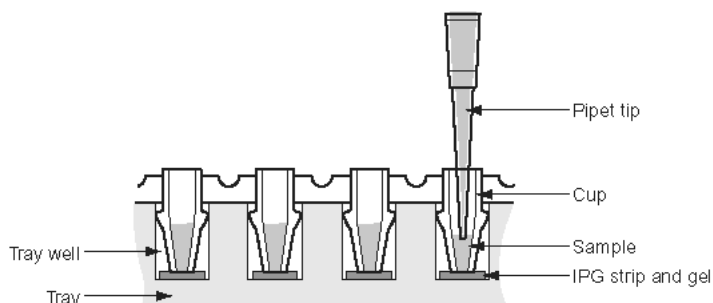


Fig 8. Side view of cup loading tray with IPG strips, cups, and sample.

Note: The sample should be prepared in a solution containing urea, a nonionic or zwitterionic detergent, carrier ampholytes, and a reducing agent. The optimal buffer composition and protein load will be sample-dependent. See section 6 for recommended buffers and for more information on sample buffer composition.

Optional: The integrity of the seal between the gel surface and the sample cup can be verified by adding some rehydration buffer to the cup prior to loading the sample. When a leakproof seal is confirmed, carefully remove the rehydration buffer and replace with sample.

14. Overlay each sample with mineral oil. Carefully pipet the oil into the sample cup so that it floats on top of the sample.
15. Completely overlay each IPG strip with mineral oil.
16. Carefully place the cup loading tray with the properly positioned electrodes and sample cups onto the temperature-controlled platform of the PROTEAN IEF cell. Close the cover. Program the PROTEAN IEF cell using the appropriate protocol in section 4. For all IPG strip lengths, use the default cell temperature of 20ºC, with a maximum current of 50 µA/IPG strip. Refer to the PROTEAN IEF Cell Instruction Manual for details on entering run parameters.

    Press START to initiate the electrophoresis run.
17. When the electrophoresis run has been completed, remove the IPG strips from the cup loading tray and transfer them gel side up into a new clean, dry disposable rehydration/equilibration tray which matches the length of the IPG strip. Hold the IPG strips vertically with forceps and let the mineral oil drain from the IPG strip for ~5 sec before transfer. Maintain the IPG strips in the same order as in the cup loading tray.
18. If you are not proceeding directly to the equilibration step (see Section 5), cover the tray containing the IPG strips, wrap it in plastic wrap, and place in a –70ºC freezer for storage.

Section 4
Focusing Conditions

Focusing conditions will vary with sample composition, sample complexity, and IPG pH range. The following parameters are recommended guidelines. The current should not exceed 50 µA/IPG strip (PROTEAN IEF default setting). The ramping protocols below have been optimized for focusing in the PROTEAN IEF cell, which features precise current and voltage control. The voltage will be limited since the current is maintained at 50 µA/IPG strip. This allows sufficient conditioning of the sample before higher voltages are applied. The total time required for ramping will therefore depend on the sample and sample buffer composition. The streamlined one-step methods for pH ranges 3–10, 3–10 NL, 4–7, 5–8, 3–6, and 7–10 replace the three-step methods in previous editions of this manual; however, the three-step methods can be used to achieve similar results.

Program the PROTEAN IEF cell using the following conditions.

ReadyStrip pH 3–10, 3–10 NL, 4–7, 5–8 Focusing Conditions

	Start Voltage	End Voltage	Volt-Hours	Ramp	Temperature
7 cm	0 V	4,000 V	8–10,000 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

Notes: 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).

Only the final voltage is entered when programming the PROTEAN IEF cell.

ReadyStrip pH 3–6 Focusing Conditions

	Start Voltage	End Voltage	Volt-Hours	Ramp	Temperature
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

Notes: 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). The lower final voltage will also increase total run time. It is recommended to not mix pH 3–6 IPG strips with other IPG strips during the focusing step.

Enhanced resolution and separation of proteins may be achieved using the Bio-Rad cup loading tray (catalog #165-4050) with sample application at the cathode (-) end of the IPG strip (refer to the Instruction Manual, Cup Loading Tray for the PROTEAN IEF Cell, (4006216), for further details).

ReadyStrip pH 7–10* Focusing Conditions

	Start Voltage	End Voltage	Volt-Hours	Ramp	Temperature
7 cm	0 V	4,000 V	8–15,000 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

Notes: 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). The lower final voltage will also increase total run time. It is recommended not to mix pH 7–10 IPG strips with other IPG strips during the focusing step.

Only the final voltage is entered when programming the PROTEAN IEF cell.

*To ensure success with basic range IPG strips, it is strongly recommended to perform two additional steps. 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. Disulfide bond formation is more problematic with basic range proteins. The second additional step is to use cup loading when loading samples for isoelectric focusing. For more information refer to ReadyPrep Reduction-Alkylation Kit Manual (4110063) and section 3.3 of this manual.

ReadyStrip pH 3.9–5.1, and 4.7–5.9 Focusing Conditions*

	Start Voltage	End Voltage	Set Time	Ramp	Temperature
Step 1					
All lengths	0 V	250 V	15 min	Rapid	20°C
	Start Voltage	**End Voltage**	**Set Time**	**Ramp**	**Temperature**
Step 2					
7 cm	250 V	4,000 V	1 hr	Slow	20°C
11 cm	250 V	8,000 V	1 hr	Slow	20°C
17 cm and 18 cm	250 V	10,000 V	2 hr	Slow	20°C
24 cm	250 V	10,000 V	2 hr	Slow	20°C
	Start Voltage	**End Voltage**	**Volt-Hours**	**Ramp**	**Temperature**
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	20–30,000 V-hr	Rapid	20°C
17 cm and 18 cm	10,000 V	10,000 V	30–45,000 V-hr	Rapid	20°C
24 cm	10,000 V	10,000 V	60–90,000 V-hr	Rapid	20°C

Notes: Only the final voltage is entered when programming the PROTEAN IEF cell.

***Enhanced resolution and separation of proteins may be achieved using the Bio-Rad cup loading tray (catalog #165-4050) (refer to the Instruction Manual, Cup Loading Tray for the PROTEAN IEF Cell, (4006216), for further details).

ReadyStrip pH 5.5–6.7, and 6.3–8.3 Focusing Conditions*

	Start Voltage	End Voltage	Time	Ramp	Temperature
Step 1					
All lengths	0 V	250 V	15 min	Rapid	20°C
	Start Voltage	**End Voltage**	**Time**	**Ramp**	**Temperature**
Step 2					
7 cm	250 V	4,000 V	1 hr	Slow	20°C
11 cm	250 V	8,000 V	1 hr	Slow	20°C
17 cm and 18 cm	250 V	10,000 V	2 hr	Slow	20°C
24 cm	250 V	10,000 V	2 hr	Slow	20°C
	Start Voltage	**End Voltage**	**Volt-Hours**	**Ramp**	**Temperature**
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	45–60,000 V-hr	Rapid	20°C
24 cm	10,000 V	10,000 V	60–90,000 V-hr	Rapid	20°C

Notes: Only the final voltage is entered when programming the PROTEAN IEF cell.

*To ensure success with basic range IPG strips, it is strongly recommended to perform two additional steps. 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. Disulfide bond formation is more problematic with basic range proteins. The second additional step is to use cup loading when loading samples for isoelectric focusing. For more information refer to ReadyPrep Reduction-Alkylation Kit manual (4110063) and section 3.3 of this manual.

Section 5
Equilibration and SDS-PAGE

Prior to running the second dimension it is necessary to equilibrate the IPG strips in SDS-containing buffers. The 2-step equilibration also ensures that cysteines are reduced and alkylated, which minimizes or eliminates vertical streaking that may be visible after staining of the second dimension gels. Equilibration Buffer I contains DTT which reduces sulfhydryl groups, while Equilibration Buffer II contains iodoacetamide which alkylates the reduced sulfhydryl groups.

SDS-PAGE Equilibration Buffer I (With DTT):
6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol, 2% (w/v) DTT

SDS-PAGE Equilibration Buffer II (With Iodoacetamide):
6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol, 2.5% (w/v) iodoacetamide

1. If the IPG strips were stored at –70ºC, they should be removed from the freezer and placed onto the lab bench to thaw at this time. The IPG strips require 10–15 min to thaw. It is best not to leave the thawed IPG strips for longer than 15–20 minutes as diffusion of the proteins can result in reduced sharpness of the protein spots.
2. Prepare the equilibration buffers. This is most conveniently done using pre-made concentrates (Equilibration Buffer I catalog #163-2107, Equilibration Buffer II catalog #163-2108).

   Pre-made equilibration buffer I contains DTT and is reconstituted by the addition of 13.35 ml of the supplied 30% glycerol solution to give 20 ml of equilibration buffer. Each bottle contains a stirbar. Place the bottle onto a stirplate and mix until all the solids have completely dissolved. This should take less than 5 min.

Pre-made equilibration buffer II contains neither DTT nor iodoacetamide and is prepared by the addition of 13.35 ml of the supplied 30% glycerol solution and 0.5 g of iodoacetamide (catalog #163-2109) to give 20 ml of equilibration buffer. Dissolve the components by magnetic stirring as described above for equilibration buffer I.

3. Remove the mineral oil from the IPG strips by placing them (gel side up) onto a piece of dry filter paper and blotting with a second piece of wet filter paper (see section 3.1, Step 10)

4. Using the table below as a guide, add the indicated volume of equilibration buffer I with DTT to an equilibration/rehydration tray, using one channel per IPG strip. Transfer the blotted IPG strips (gel side up) into the equilibration/rehydration tray.

	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

5. Place the tray on an orbital shaker and gently shake for 10 min. Select a slow shaker speed to prevent the buffer from sloshing out of the tray.

6. At the end of the 10 min incubation, discard the used equilibration buffer I by carefully decanting the liquid from the tray. Decanting is best carried out by pouring the liquid from the square side of the equilibration/rehydration tray until the tray is vertical. Take care not to pour out the liquid too quickly at first as the IPG strips may slide out. When most of the liquid has been decanted, flick the tray a couple of times to remove the last few drops of equilibration buffer I.

7. Using the table above, add the indicated volume of equilibration buffer II with iodoacetamide to each IPG strip. Return the tray to the orbital shaker for 10 min.

Note: The second equilibration with equilibration buffer II is not necessary in all circumstances, particularly when the sample has been reduced and alkylated. If the second equilibration step is not performed, prolong the duration of the first equilibration step to 20 min.

8. During the incubation, melt the overlay agarose (catalog #163-2111) solution in a microwave oven.
9. Discard the equilibration buffer II with iodoacetamide by decanting at the end of the incubation period as described in step 6 above.
10. Fill a 100 ml graduated cylinder or a tube that is the same length as or longer than the IPG strip length with 1x Tris-glycine-SDS (TGS) running buffer. Use a Pasteur pipet to remove any bubbles on the surface of the buffer.
11. Finish preparing the SDS-PAGE gels by blotting away any excess water remaining inside the IPG well using Whatman 3 MM or similar blotting paper.
12. Place an SDS-PAGE gel in an AnyGel™ stand. For ideal IPG strip loading, use the 6-row AnyGel stand (catalog #165-5731) to properly position the gel cassette at an angle in front of the stand, as shown in Figure 10 (refer to the AnyGel Stands Instruction Manual, bulletin 4006207). Remove an IPG strip from the disposable rehydration/equilibration tray and dip briefly into the graduated cylinder containing 1x TGS running buffer, as

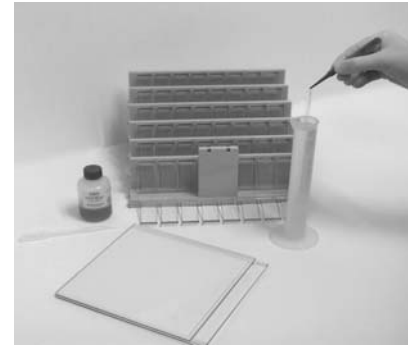


Fig. 9. Rinsing the IPG Strip in 1x TGS running buffer.

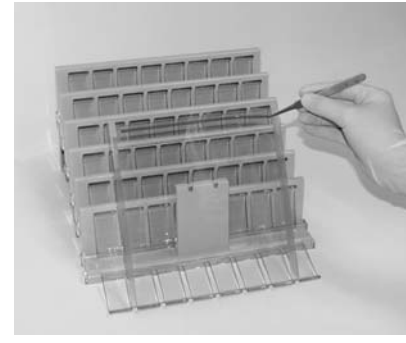


Fig. 10. Placing the IPG strip gel side up on the back plate of the SDS-PAGE gel.

shown in Figure 9. Lay the strip, with the gel side towards you, onto the back plate of the SDS-PAGE gel above the IPG well (see Figure 10). Repeat this process for any remaining IPG strips.

13. Use a Pasteur pipet or a disposable plastic transfer pipet (catalog #223-9522) and place overlay agarose solution (catalog #163-2111) into the IPG well of the gel (see Figure 11). For gels run in the PROTEAN Plus Dodeca cell, use PROTEAN® Plus overlay agarose (catalog #163-2092).

14. Using the forceps, or a spatula, carefully push the IPG strip into the well as shown in Figure 12, taking care not to trap any air bubbles beneath the IPG strip. When pushing the IPG strips with the forceps, be sure that the forceps are pushing on the plastic backing of the IPG strip and not the gel matrix. Alternatively, the IPG strips can be pushed in contact with the second-dimension gel surface prior to applying the agarose solution over the IPG strip (this alternative method is often necessary with second-dimension gels ≥ 20 cm in width, as the agarose solidifies before the IPG strip can be properly inserted).

15. Stand the gel(s) vertically by placing them in the electrophoresis cell (Criterion™ system) or in the AnyGel stand. Allow the agarose to solidify for 5 min before proceeding.

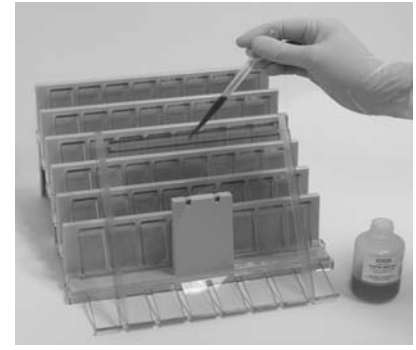


Fig. 11. SDS-PAGE gel with IPG strip held vertically in rack. Pipetting overlay agarose into the IPG well.

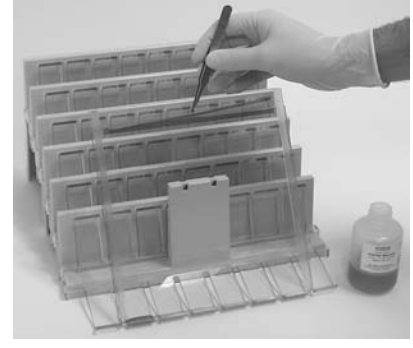


Fig. 12. Sliding IPG strips into IPG well filled with melted overlay agarose.

16. Mount the gel(s) into the electrophoresis cell following the instructions provided with the apparatus.
17. Fill the reservoirs with 1x TGS running buffer and begin the electrophoresis. The table below lists the electrophoresis run conditions for each of Bio-Rad's SDS-PAGE formats. The migration of the Bromophenol Blue, present in the overlay agarose solution, is used to monitor the progress of the electrophoresis.

	Strip Length				
	7 cm	**11 cm**	**17 cm**	**17 cm or 18 cm**	**24 cm**
Electrophoresis cells	Mini-PROTEAN® 3 and Mini-PROTEAN Dodeca™	Criterion and 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	35 min	55 min	5.5 hr	6–8 hr*	6–8 hr*

*For handcast gels. For PROTEAN Plus precast gels, follow instructions provided with the gels.

Section 6
General Guidelines

Cell lysis: Unless the sample to be analyzed is a cell-free fluid such as serum, the first step of sample preparation will be cell lysis or tissue disruption. The sample is usually rapidly lysed directly into a strongly denaturing solution in order to inactivate any enzymes that might degrade or modify proteins. As an additional precaution, protease inhibitors can be added to the sample/lysis buffer. The method of lysis will vary greatly depending on the source of the cells. Follow standard procedures for cell growth and tissue harvesting and a standard method for physical cell lysis appropriate for the cell or tissue type. Use relative amounts of sample cells or tissue that are estimated to yield 1–20 mg/ml of protein. Substances that can potentially interfere with IEF separation, such as salts, should be avoided if possible. Lysis conditions are very important to the success of the extraction. Proper conditions for thorough lysis should be determine empirically. Several texts and Internet sites can be consulted for details of cell culturing and cell lysis for a wide variety of cell types. For example, http://www.expasy.org/ch2d/protocols/protocols.fm1.html and references 2–7 are also valuable sources of information on sample preparation.

1) **Animal tissues:** Animal tissues generally have low levels of interfering substances and can be prepared relatively simply for 2-D electrophoresis. A good method is to freeze the tissue in liquid nitrogen immediately after harvest. The tissue is then ground to a frozen powder in a mortar and pestle. An appropriate sample buffer is added and the proteins are allowed to solubilize. Insoluble material is removed by centrifugation.

2) **Tissue culture cells:** Preparation of samples from tissue culture cells is also relatively straightforward. The most important consideration is to minimize carryover of exogenous proteins, salts and other undesirable components from the culture medium. Following harvest by centrifugation, the cells should be washed with a low-salt, isotonic buffer such as 10 mM Tris-HCl, 250 mM sucrose pH 7.0. It is

important to remove all of the wash buffer from the cell pellet prior to the addition of sample buffer. Sonication or homogenization can be used to achieve complete lysis.

3) **Bacterial cells:** The major issue with bacterial extracts is their high nucleic acid to protein ratio. DNA and RNA can interfere with focusing and the presence of DNA can render the sample too viscous to work with effectively. Bacterial lysates are often treated with nucleases to prevent these problems. One method is to add a one-tenth volume of a solution containing 1 mg/ml DNase I, 0.25 mg/ml RNase A and 50 mM $MgCl_2$ to the sample. High concentrations of urea inactivate these enzymes, so this treatment should be applied prior to addition of urea.

4) **Plant tissues:** Plant tissues can be prepared by freezing and grinding with liquid nitrogen as described above for animal tissues. However, plant tissues tend to contain high levels of interfering substances and are relatively dilute sources of protein. Plant extracts should therefore be treated with the ReadyPrep™ 2-D Cleanup kit (catalog #163-2130) to remove interfering substances and to concentrate the sample.

5) **Yeast cells:** Yeast cells have tough cell walls and require vigorous lysis conditions. They also contain high levels of protease which should be inactivated by lysis directly into an SDS-containing sample buffer. One method is to lyse the cells by sonication or vortexing with glass beads into a buffer containing 1% SDS, 50 mM Tris-HCl at pH 7.0. Following centrifugation, the lysate is diluted into a 5-fold excess of IPG sample buffer containing urea and CHAPS.

Sample Buffer

Proteins in the sample must be fully denatured, reduced and solubilized for effective first-dimension IEF separation. Sample buffers are prepared with several components to achieve this. A wide variety of different agents are employed, but sample buffers for 2-D electrophoresis always contain the following: 1) a neutral chaotrope, which is either urea or a mixture of urea and thiourea, 2) a reductant capable of reducing disulfide bonds (e.g. DTT, Tributylphosphine), 3) a neutral or zwitterionic detergent (e.g. CHAPS, Triton X-100). The anionic detergent SDS is often used due to its ability to rapidly solubilize protein while simultaneously

inactivating proteases. However, if SDS is used, the final concentration of SDS should be 0.25% or lower and a neutral or zwitterionic detergent should be present in at least an 8-fold excess over the SDS. If other interfering substances must be used during sample preparation, or if the sample contains high endogenous levels of interfering substances, they may be removed using the ReadyPrep™ 2-D Cleanup kit (catalog #163-2130). In many cases, a sample buffer identical in composition to the rehydration buffer may be used. Total salt concentration in the sample should not exceed 10 mM. Samples containing urea should never be heated above 30ºC.

Bio-Rad offers four premixed sample buffers. These are listed in order of increasing solubilizing power:

1) ReadyPrep 2-D starter kit rehydration/sample buffer, catalog #163-2106 (8 M urea, 2% CHAPS, 50 mM DTT, 0.2% Bio-Lyte® 3/10 ampholyte, 0.001% Bromophenol Blue)
2) ReadyPrep sequential extraction kit reagent 2, catalog #163-2103 (8 M urea, 4% CHAPS, 40 mM Tris, 0.2% Bio-Lyte 3/10 ampholyte)[8]
3) ReadyPrep sequential extraction kit reagent 3, catalog #163-2104 (5 M urea, 2 M thiourea, 2% CHAPS, 2% SB 3–10, 40 mM Tris, 0.2% Bio-Lyte 3/10 ampholyte)[8]
4) ReadyPrep 2-D rehydration/sample buffer 1, catalog #163-2083 (7 M urea, 2 M thiourea, 1% (w/v) ASB-14, 40 mM Tris, 0.001% Bromophenol Blue)

Sample Pre-fractionation

In many cases, it is desirable to lower the overall complexity of the protein sample and only analyze a subset of all of the proteins present. There are many means to accomplish this, including subcellular fractionation and fractionation by differential solubility. Bio-Rad offers a number of sample preparation kits for sample pre-fractionation. These include: ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear) (catalog #163-2089), ReadyPrep Protein Extraction Kit (Membrane I) (catalog #163-2088), ReadyPrep Protein

Extraction Kit (Signal) (catalog #163-2087), ReadyPrep Protein Extraction Kit (Soluble/Insoluble) (catalog #163-2085), ReadyPrep Protein Extraction Kit (Membrane II) (catalog #163-2084), ReadyPrep Sequential Extraction Kit (catalog #163-2100).

Rehydration Buffer

A useful rehydration buffer for many samples is given below. Ideally, the composition of this buffer should be optimized for each individual sample. Refer to the guidelines below when optimizing the rehydration buffer.

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 buffer composition for each sample type is best determined empirically.

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

1) Urea — Urea maintains sample proteins in their denatured state and keeps them soluble. A concentration of 8 M is recommended, but concentrations up to 9.8 M may be required for complete sample solubilization. Thiourea can also be used to improve the solubility of samples.[9,10] The total concentration of urea plus thiourea should be in the range 7–9 M. Prepare urea solutions just prior to use or store at –20°C in aliquots and thaw prior to use. Discard unused portions.

Note: If the sample was prepared using thiourea, thiourea should also be present in the rehydration solution. Otherwise, proteins that require thiourea for solubility will precipitate.

2) **Carrier ampholytes** — Carrier ampholytes mixtures (sometimes referred to as IPG buffer) improve protein solubility and ensure uniform conductivity during IEF without altering the pH gradient of the IPG strip. Bio-Lyte 3/10 is recommended for IPG strips with broad and narrow pH ranges. Specific ampholyte mixtures, available as 100x concentrates, are recommended for micro ranges; and pH 7–10 IPG strips. Below is a table of recommended Bio-Lyte buffers by IPG strip pH range.

Recommended Bio-Lyte mixtures by IPG strip pH range

IPG Strip	Buffer	IPG Strip	Buffer
3–10	Bio-Lyte 3–10	3.9–5.1	ReadyStrip 3.9–5.1 buffer*
3–10 NL	Bio-Lyte 3–10		
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*
7–10	ReadyStrip 7–10 buffer*		

* 100x ReadyStrip buffers when diluted to 1x in the final sample yield a concentration of 0.2% Bio-Lyte.

3) **Detergents** — Detergents aid in protein solubility and minimize protein aggregation. Use only nonionic or zwitterionic detergents, including Triton X-100, CHAPS, SB 3–10, and ASB-14. Total detergent concentration is usually in the range 0.5–4%.

Note: Zwitterionic detergents of the SB series are not soluble in concentrated urea solutions. If these detergents are used, the concentration of urea in the rehydration solution should be lowered to 5 M.

Note: The effects of detergents are not additive and can be unpredictable in mixtures. The use of mixed detergents is generally not recommended.

4) **Reducing agents** — Reducing agents are included in rehydration solutions in order to maintain protein sulfhydryls in their reduced state and to prevent oxidative crosslinking through disulfide bonds. DTT (10–100 mM) is the current standard; however, DTT is negatively charged at basic pH. It will therefore migrate out of the basic end of the pH range, allowing basic proteins to oxidize and become insoluble. If basic proteins are to be resolved, this problem should be circumvented by treating the sample with the ReadyPrep Reduction Alkylation Kit (catalog #163-2090). This treatment blocks protein sulfhydryls, preventing their oxidation to disulfides.

5) **Protein load** —The total amount of protein to load per IPG strip will vary depending on the sample, the pH range and length of the IPG strip, and the detection system used. In some cases, overloading of protein is acceptable to reveal less-abundant proteins of interest. Below is a guideline for protein loads that generally gives acceptable 2-D patterns. Use lower amounts for silver or SYPRO Ruby protein staining and higher amounts for Coomassie Blue staining. In general, the maximum that can be loaded onto each IPG strip is 500 µg for 7 cm, 1 mg for 11 cm, 3 mg for 17 cm/18 cm, and 4 mg for 24 cm IPG strips.

Recommended Range of Protein Loads for IPG Strips

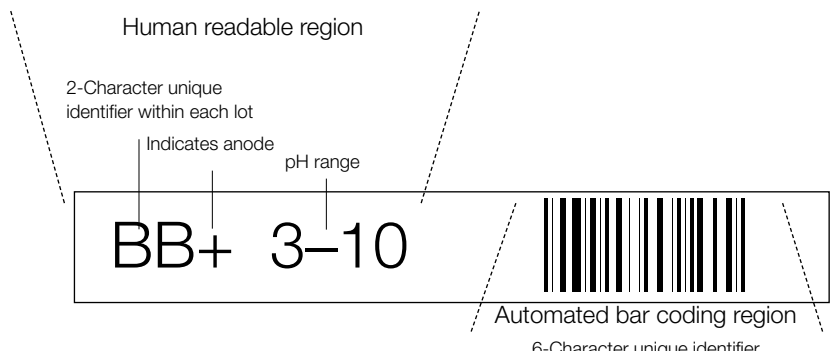
IPG strip length	7 cm	11 cm	17 cm	18 cm	24 cm
Rehydration volume per strip	125 µl	200 µl	300 µl	315 µl	450 µl
Protein load					
Silver stain	5–20 µg	20–50 µg	50–80 µg	50–80 µg	80–150 µg
Protein load					
Coomassie G-250	50–100 µg	100–200 µg	200–400 µg	200–400 µg	400–800 µg

Sample calculation: If you precipitate 100 µg of protein and are going to run 7 cm pH 3–10NL IPG strips (125 µl per IPG strip) and silver stain the 2-D gels, then you may want to solubilize the protein pellet in ~900 µl of rehyrdration/sample buffer, which is enough to rehydrate about seven 7 cm IPG strips (~14 µg/IPG strip). However, if you are planning to use a 24 cm pH 3–10NL IPG strip, then you may want to solubilize the protein pellet in 450 µl of rehyrdration/sample buffer, which is enough to rehydrate one 24 cm IPG strip (100 µg/IPG strip). In this simple example, sample complexity and IPG strip pH range were not addressed. As a general rule, increased protein loads may be required for micro-range IPG strips and for samples of higher protein complexity.

Section 7
Product Information

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 QuickScan 6000/6000plus reader.* 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.



*PSC Scanning, Inc., www.pscnet.com, 959 Terry St., Eugene, OR, 97402, USA Tel: 1-800-547-2507

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

*Nonlinear gradient

Catalog #	Description
Sample Preparation Kits	
163-2130	ReadyPrep 2-D Cleanup Kit, 50 preps
163-2089	ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear), 50 preps
163-2088	ReadyPrep Protein Extraction Kit (Membrane 1), 50 preps
163-2087	ReadyPrep Protein Extraction Kit (Signal), 50 preps
163-2086	ReadyPrep Protein Extraction Kit (Total Protein), 20 preps
163-2085	ReadyPrep Protein Extraction Kit (Soluble/Insoluble), 20 preps
163-2084	ReadyPrep Protein Extraction Kit (Membrane II), 10 preps
163-2090	ReadyPrep Reduction/Alkylation Kit, 100 preps
163-2100	ReadyPrep Sequential Extraction Kit, 5–15 preps

Catalog #	Description
Buffers and Reagents	
163-2094	100x Bio-Lyte 3–10 Ampholyte, 1 ml
163-2093	100x ReadyStrip 7–10 Buffer, 1 ml
163-2098	100x ReadyStrip 3.9–5.1 Buffer, 1 ml
163-2097	100x ReadyStrip 4.7–5.9 Buffer, 1 ml
163-2096	100x ReadyStrip 5.5–6.7 Buffer, 1 ml
163-2095	100x ReadyStrip 6.3–8.3 Buffer, 1 ml
163-2105	ReadyPrep 2-D Starter Kit, 1 kit
163-2106	ReadyPrep 2-D Starter Kit Rehydration/Sample Buffer, 1 vial
163-2103	ReadyPrep Sequential Extraction Kit Reagent 2, 1 vial
163-2104	ReadyPrep Sequential Extraction Kit Reagent 3, 1 vial
163-2083	ReadyPrep 2-D Rehydration/Sample Buffer 1, 10 ml
163-2107	ReadyPrep 2-D Starter Kit Equilibration Buffer I, with DTT, 1 vial
163-2108	ReadyPrep 2-D Starter Kit Equilibration Buffer II, without DTT or iodoacetamide, 1 vial
163-2129	Mineral Oil, 500 ml
163-2111	ReadyPrep Overlay Agarose, 50 ml
163-2092	PROTEAN Plus Overlay Agarose, 125 ml
161-0731	Urea, 1 kg
161-0716	Tris, 1 kg
161-0611	DTT, 5 g
163-2101	Tributylphosphine (TBP), 200 mM, 0.6 ml
163-2109	Iodoacetamide, 30 g
161-0460	CHAPS, 1 g
161-0732	10x Tris/Glycine/SDS, 1 L
161-0772	10x Tris/Glycine/SDS, 5 L cube

Catalog #	Description
PROTEAN IEF Cell and Accessories	
165-4000	PROTEAN IEF System, complete, 1
165-4001	PROTEAN IEF Cell, basic unit, 1
165-4030	7 cm Focusing Tray With Lid, 1
165-4020	11 cm Focusing Tray With Lid, 1
165-4010	17 cm Focusing Tray With Lid, 1
165-4040	18 cm Focusing Tray With Lid, 1
165-4042	24 cm Focusing Tray With Lid, 1
165-4071	Electrode Wicks, 500 precut per pack
165-4035	Disposable Rehydration/Equilibration Tray With Lid, 7 cm, 25
165-4015	Disposable Rehydration/Equilibration Tray With Lid, 17 cm, 25
165-4025	Disposable Rehydration/Equilibration Tray With Lid, 11 cm, 25
165-4041	Disposable Rehydration/Equilibration Tray With Lid, 18 cm, 25
165-4043	Disposable Rehydration/Equlibration Tray With Lid, 24 cm, 25
165-4050	Cup Loading Tray, 1
165-4051	Large Replacement Cups, 150 µl, 1 pack (120 count)
165-4052	Small Replacement Cups, 100 µl, 1 pack (120 count)
165-4053	Replacement Movable Electrodes, 1 pair
165-4054	Replacement Cup Loading Tray Base
165-5131	AnyGel Stand, 6-row, holds 6 PROTEAN Plus gels, 12 Criterion gels, or 18 mini gels; includes instructions
345-9920	Criterion™ Staining and Blotting Trays (12 Trays)

Section 8
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10. Rabilloud T et al., Improvement of the solubilization of proteins in two-dimensional electrophoresis with immobilized pH gradients, Electrophoresis 18, 307–316 (1997)

Catalog Number
163-2099

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Life Science Group

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Finland 358 (0)9 804 2200 **France** 01 47 95 69 65 **Germany** 089 318 84-177
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Israel 03 951 4127 **Italy** 39 02 216091 **Japan** 03-5811-6270
Korea 82-2-3473-4460 **Latin America** 305-894-5950
Mexico 52 5 534 2552 to 54 **The Netherlands** 0318-540666
New Zealand 64-9-4152280 **Norway** 47-23-38-41-30 **Portugal** 351-21-472-7700
Russia 7 095 721 1404 **Singapore** 65-2729877
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Sweden 46 (0)8-55 51 27 00 **Switzerland** 061 717-9555 **United Kingdom** 0800-181134

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