
ReadyPrep™ 2-D Starter Kit

Instruction Manual

Catalog Number
163-2105

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Section 1

Introduction

The ReadyPrep™ 2-D starter kit was designed as a single-use kit to familiarize first-time users with the utilization of the Bio-Rad PROTEAN® IEF cell and ReadyStrip™ IPG strips. The kit provides all the reagents necessary, including a protein control, to successfully perform two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). The kit contains sufficient reagents to perform 2-D PAGE of at least 6 samples with any of the three SDS-PAGE formats Bio-Rad offers. The amount of sample applied to each gel is sufficient so that a large number of protein spots can be readily detected when using either Bio-Safe™ Coomassie* stain or Coomassie Brilliant Blue R-250 stain. This manual provides instructions on how to perform 2-D PAGE with ReadyStrip IPG strips using the Bio-Rad PROTEAN IEF cell and the control *E. coli* protein sample.

Section 2

Kit Components

ReadyPrep *E. coli* Protein Sample. One vial. Lyophilized. Each vial of reconstituted ReadyPrep *E. coli* protein sample contains 2.7 mg of total protein at a concentration of 1.35 mg/ml.

ReadyPrep Rehydration/Sample Buffer. One vial. Lyophilized. Each vial of reconstituted ReadyPrep rehydration buffer contains 10 ml of 8 M urea, 2% CHAPS, 50 mM dithiothreitol (DTT), 0.2% (w/v) Bio-Lyte® 3/10 ampholytes, and Bromophenol Blue (trace).

Nanopure Water. One bottle containing 15 ml of sterile nanopure water.

Equilibration Buffer I. Two vials, each containing a stirbar. Lyophilized. Each vial of reconstituted equilibration buffer I contains 20 ml of 6 M urea, 2% SDS, 0.375 M Tris-HCl (pH 8.8), 20% glycerol, and 2% (w/v) DTT.

Equilibration Buffer II. Two vials, each containing a stirbar. Lyophilized. Each vial of reconstituted equilibration buffer II contains 20 ml of 6 M urea, 2% SDS, 0.375 M Tris-HCl (pH 8.8), and 20% glycerol.

30% Glycerol Solution. One bottle containing 70 ml of sterile 30% (v/v) glycerol.

Iodoacetamide. Two vials. Each vial contains 0.5 gr of an ultrapure grade of iodoacetamide.

*Coomassie is a trademark of Imperial Chemical Industries PLC.

Overlay Agarose. One bottle containing 50 ml of 0.5% low melting point agarose in 25 mM Tris, 192 mM glycine, 0.1% SDS, and a trace of Bromophenol Blue.

Instruction Manual. One.

Note:

CHAPS is 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, a zwitterionic detergent.

Bio-Lyte® 3/10 ampholytes is a mixture of carrier ampholytes, pH 3–pH 10.

Other Materials Needed

- PROTEAN IEF cell
- ReadyStrip IPG strips, pH 4-7 (7 cm, 11 cm, or 17 cm)
- IEF focusing tray with lid (same size as IPG strips)
- Electrode wicks, precut
- Blotting filter papers
- Mineral oil
- Forceps
- Pipets for volumes ranging from 4-1000 µl
- Stirplate
- Plastic (Saran) wrap
- 8-16% SDS-PAGE gels (Ready Gel®, Criterion, or PROTEAN II Ready Gel precast gels)
- SDS-PAGE electrophoresis cell (Mini-PROTEAN® 3, Ready Gel, Criterion, or PROTEAN II XL cell)
- Power supply appropriate for SDS-PAGE system
- Tris/glycine/SDS running buffer
- SDS-PAGE protein stain (Bio-Safe Coomassie or Coomassie Brilliant Blue R-250)
- IEF protein stain (IEF stain or Bio-Safe Coomassie)
- Destain solution (40% methanol, 10% acetic acid)
- Disposable rehydration/equilibration trays with lid (same size as IPG strips)
- SDS-PAGE gel staining trays
- 100 ml graduated cylinders
- High-purity water

Section 3

Storage

Store the entire kit at 4°C.

Section 4

Instructions for Use

Introduction

This 2-D starter kit allows you to successfully separate a complex protein extract by 2-D PAGE using IPG strips and the Bio-Rad PROTEAN IEF cell. The buffer formulations as well as the following protocol have been optimized for the *E. coli* protein sample included in the kit. Before beginning, decide which length IPG strips you wish to use and how many you wish to focus. The kit contains sufficient reagents for the separation of the *E. coli* protein sample on six 17 cm IPGs, ten 11 cm IPGs or sixteen 7 cm IPGs. The user has the flexibility to choose among three SDS-PAGE formats (7 cm IPG strips and Mini-PROTEAN® 3 Ready Gel precast gels, 11 cm IPG strips and Criterion™ gels or 17 cm IPG strips and PROTEAN® II Ready Gel® precast gels). The mini and Criterion formats have the advantage that the entire 2-D electrophoresis process can be completed within a 24 hour period. The flow chart in Figure 1 can be used to estimate the amount of time necessary to complete each of the steps of the 2-D process.

The instructions below describe the use of the *E. coli* protein sample with six IPG strips. Two of these strips will be stained after the first dimension to illustrate the successful completion of the IEF step. The remaining IPGs will be carried through the second-dimension separation. The SDS-PAGE gels can be stained with either Bio-Rad's Bio-Safe Coomassie stain or with Coomassie Brilliant Blue R-250 stain.

The ReadyPrep 2-D starter kit has been optimized for pH 4-7 ReadyStrip IPG strips and either Bio-Safe Coomassie or Coomassie Brilliant Blue R-250 stain. Strips of pH 5-8 can be used but the results will not match the enclosed gel images. Strips of pH 3-10 are not recommended unless the protein sample is further diluted into rehydration/sample buffer at least 4-fold. Other stains can also be used, but again the protein sample needs to be diluted if a more sensitive stain is used.

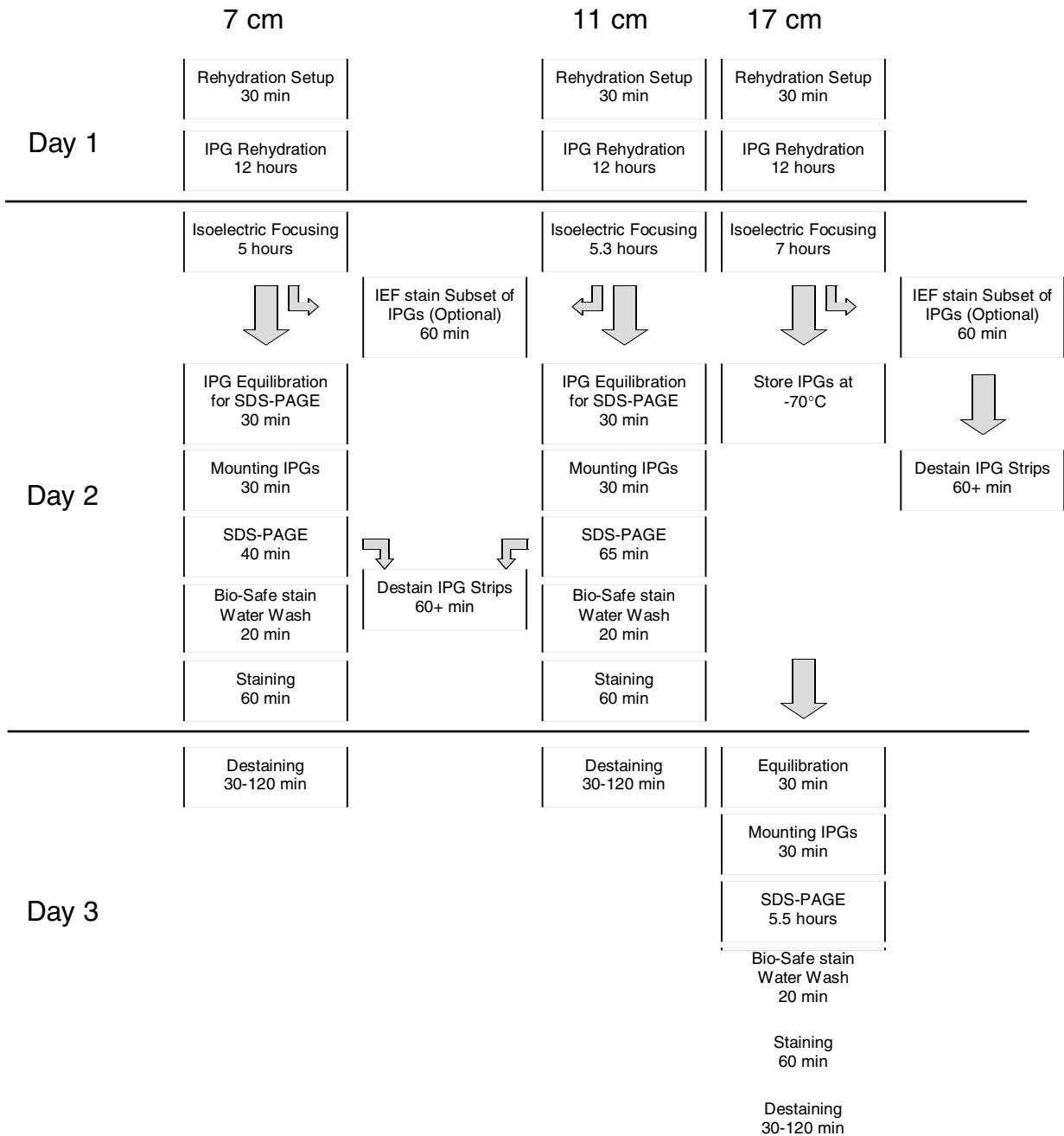


Fig. 1. Flow Chart for 2-D SDS-PAGE.

4.1 Sample Preparation

- 1 Remove the bottle of rehydration/sample buffer, the bottle of nanopure water and the vial of *E. coli* protein sample from the kit. All 3 bottles have a GREEN cap.
- 2 Remove the desired number of pH 4-7 ReadyStrip IPG strips from the -20°C freezer and set aside. (Six strips are recommended: 2 to stain after IEF, 4 to be used for second-dimension SDS-PAGE).
- 3 Place one disposable rehydration/equilibration tray of the same size as the IPG strips to be run onto the bench with the sloped end facing to the right (See Figure 2).
- 4 Remove the crimp and stopper from the bottle of rehydration/sample buffer and reconstitute the lyophilized powder by adding 6.1 ml of the nanopure water supplied with the kit. Swirl the bottle gently at intervals until all the solids are completely dissolved. The bottle will chill as the urea in the solids dissolves. The bottle can be warmed slightly in the palm of the hand to expedite this buffer reconstitution process. Take care not to warm the solution above 30°C.
- 5 Open the vial of *E. coli* protein sample and reconstitute the lyophilized protein by adding 2 ml of the freshly prepared rehydration/sample buffer. Replace the rubber stopper and gently invert the vial 4 to 6 times to completely reconstitute the *E. coli* proteins. Do NOT place the vial on ice as the urea will crystallize out of the solution.

4.2 Sample Application During Rehydration

The amount of reconstituted *E. coli* protein sample to load per IPG strip is indicated in Table 1. These quantities are sufficient to easily visualize a large array of protein spots when the second-dimension SDS-PAGE gel is stained with Bio-Safe Coomassie stain or Coomassie Brilliant Blue R-250 stain.

1. Using Table 1 below, pipet the indicated volume of the reconstituted *E. coli* protein sample as a line along the back edge of channel #1. 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 strip (See Figure 2. Sample loading of rehydration/equilibration trays).

The increased sensitivity of the Bio-Safe Coomassie stain over the R-250 stain allows you to dilute the stock of *E. coli* protein sample up to 4-fold into rehydration/sample buffer and still achieve excellent images. If you choose to stain the SDS-PAGE gels with silver stain (such as Bio-Rad's Silver Stain Plus stain, catalog # 161-0449) or a fluorescent stain (such as SYPRO* Ruby Stain, catalog # 170-3125) use a 4-fold diluted *E. coli* protein sample (diluted into rehydration/sample buffer) and the rehydration volumes per strip indicated below.

Table 1.

Strip Length	7 cm	11 cm	17 cm
Sample Volume	125 µl	185 µl	300 µl
Protein Loaded	169 µg	250 µg	405 µg

*SYPRO is a trademark of Molecular Probes, Inc.

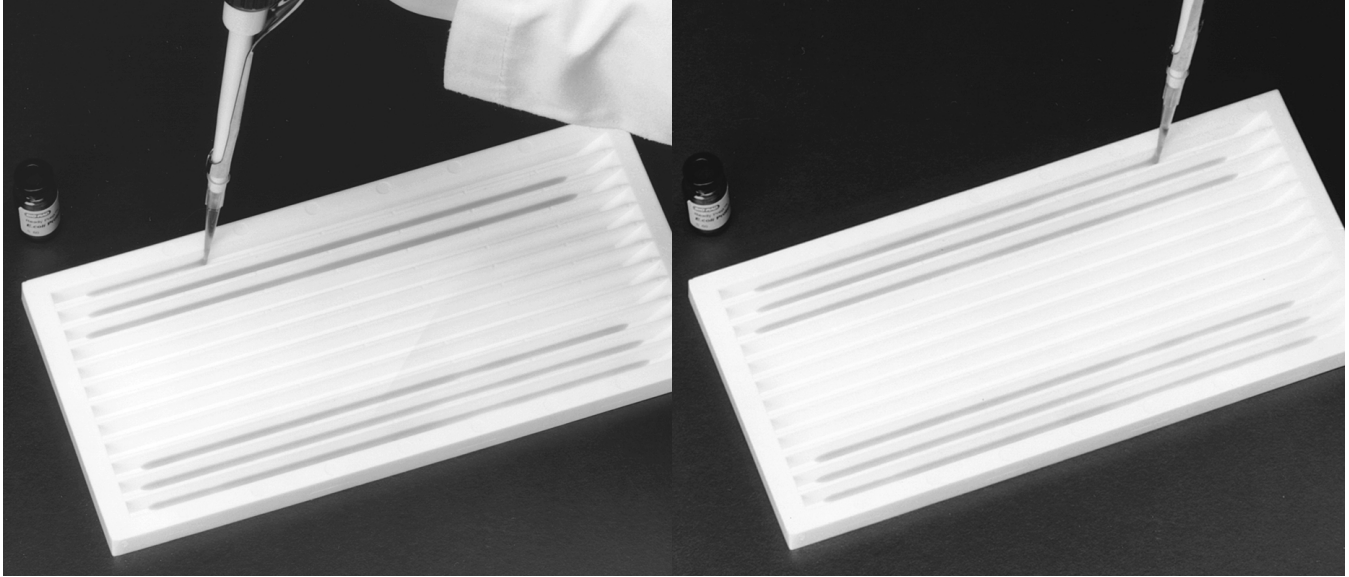


Fig. 2. Sample loading of rehydration/equilibration trays. Pipet the sample along the back edge of the tray channel except for about 1 cm at each end. Note the even distribution of the sample along the edge of the channel. The figure shows the last of six samples being pipeted in the tray.

2. Repeat this process for the remaining samples by pipetting the indicated volume of sample into adjacent channels. It is best to place samples on both sides of the tray as in Figure 2. Maintaining the same numbering system throughout the 2-D PAGE process helps to avoid confusing the samples. Also, during the IEF step when strips are placed on both sides of the focusing tray, the weight of the tray lid is evenly distributed over all the samples so contact between the IPGs and the electrode remains uniform.
3. When all the protein samples have been loaded into the rehydration/equilibration tray as pictured in Figure 2, using forceps, peel the coversheet from one of the pH 4-7 ReadyStrip IPG strips, as in Figure 3.



Fig. 3. Removing the cover sheet from the ReadyStrip IPG strip.

Gently place the strip gel side down onto the sample as illustrated in Figure 4. The “+” and “pH 4-7” should be legible and positioned at the left side of the tray. Take care not to get the sample onto the plastic backing of the 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 strip. If this happens, carefully use the forceps to lift the strip up and down from one end until the air bubbles move to the end and out from under the strip.

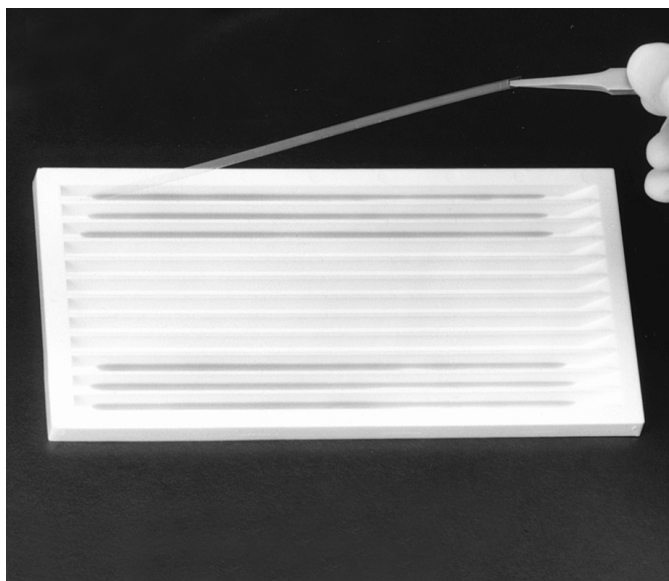


Fig. 4. Placing IPG strips gel side down onto the protein samples.

Rehydration of the IPG strips is a critical step in the 2-D process. The method shown in Figures 2-4 allows for consistently even distribution of the sample along the length of the strip. If the sample appears unevenly distributed or areas of the strip were not wetted with sample, hold the strip at one end with the forceps and slide the strip back and forth several times along the length of the channel.

- 4 Overlay each of the strips with 2 to 3 ml of mineral oil to prevent evaporation during the rehydration process. Add the mineral oil slowly, by carefully dripping the oil onto the plastic backing of the strips while moving the pipet along the length of the strip.
- 5 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.
This is the final step of day 1.

Mineral oil prevents evaporation of the sample during rehydration, thus preventing precipitation of the urea. IPG strips can be left to rehydrate for up to 1 hr before adding the oil. This simplifies overlaying the strips with oil as most of the sample is now absorbed into the strip.

4.3 Isoelectric Focusing.

- 1 Place a clean, dry PROTEAN IEF focusing tray the same size as the rehydrating IPG strips onto the lab bench.
- 2 Using forceps, place a paper wick at both ends of the channels covering the wire electrodes. Use channels with the same numbers as those used during rehydration. As shown in Figure 5, channel numbers 1, 2, 3, 10, 11, and 12 contain electrode wicks.
- 3 Pipet 8 μ l of nanopure water (provided) onto each wick to wet them as shown in Figure 6. Readjust their position if necessary.
- 4 Remove the cover from the rehydration/equilibration tray containing the IPG strips. Using forceps, carefully hold the strip vertically for about 7 to 8 seconds to allow the mineral oil to drain (Figure 7), then transfer the IPG strip to the corresponding channel in the focusing tray (maintain the gel side down) (Figure 8). Repeat for all the strips.

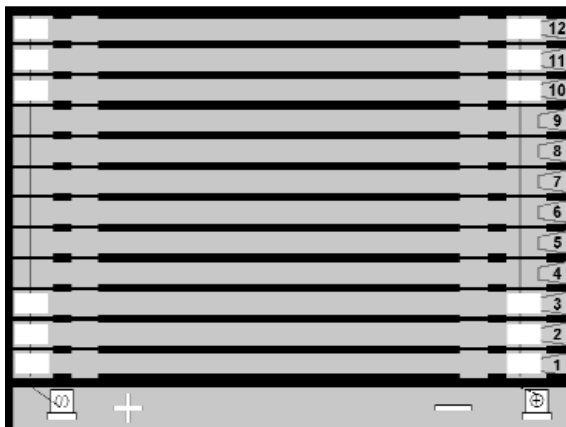


Fig. 5. Placement of paper wicks over electrode at each end of channel.

Electrode wicks are important as receptacles for salts and other non-amphoteric constituents of the sample, and they improve the quality of the results.



Fig. 6 Wetting the electrode wicks with water.

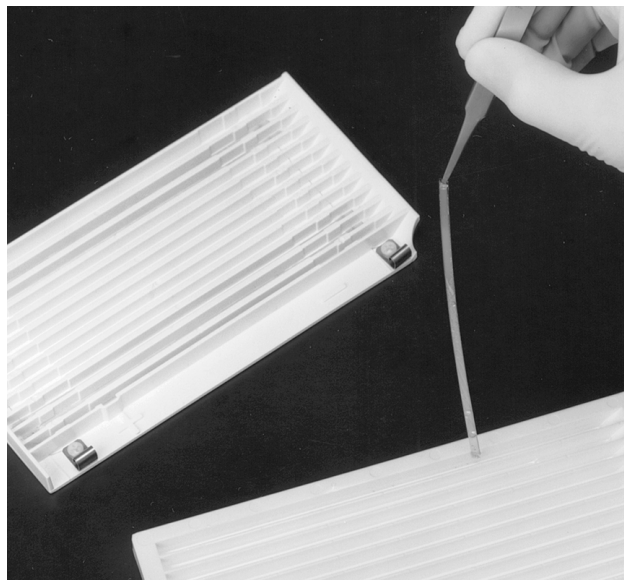


Fig. 7 Draining the oil.

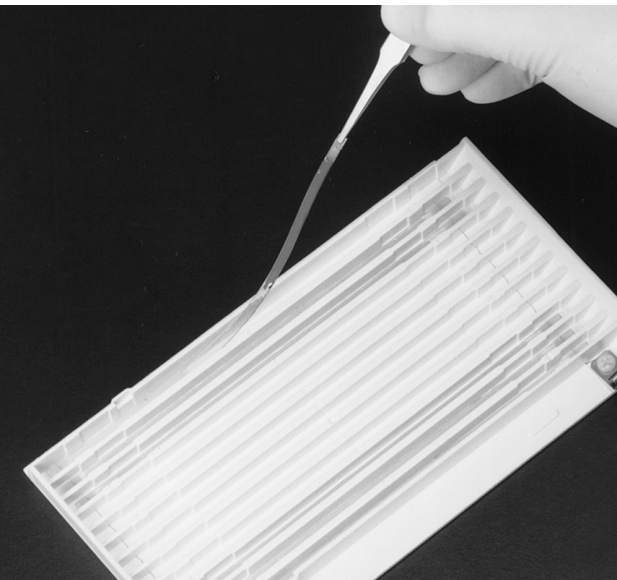


Fig. 8 Placing the ReadyStrip gel side down in the focusing tray.

Draining the oil essentially washes the outside surface of the gel, removing unabsorbed protein and resulting in reduced horizontal streaking. If desired, the disposable rehydration/equilibration tray can be cleaned, dried, and used to store strips after the completion of the first-dimension run.

- 5 Cover each IPG strip with 2 to 3 ml of fresh mineral oil. Check for, and if applicable remove, any trapped air bubbles beneath the strips. Place the lid onto the tray (positive "+" to the left when the inclined portion of the tray is on the right as in Figure 8).
- 6 Place the focusing tray into the PROTEAN IEF cell and close the cover.
- 7 Program the PROTEAN IEF cell using the appropriate 3-step protocol in Table 2. For all strip lengths, use the default cell temperature of 20°C, with a maximum current of 50 µA/strip and No Rehydration. Refer to Section 5, Appendix, or the PROTEAN IEF cell manual for details on entering the run parameters.
- 8 Press START to initiate the electrophoresis run.

Remember to observe the correct polarity during the transfer of strips into the focusing tray. The convenient "+" and "pH4-7" printed on the strips (acidic end) should be positioned at the end of the tray marked with the "+" (this is the left side of the tray when the inclined portion of the tray is on the right as in Figure 7)

Table 2.

7 cm	Voltage	Time	Volt-Hours	Ramp
Step 1	250	20 min	-----	Linear
Step 2	4,000	2 hr	-----	Linear
Step 3	4,000	-----	10,000 V-hr	Rapid
Total		5 hr	14,000 V-hr	
11 cm	Voltage	Time	Volt-Hours	Ramp
Step 1	250	20 min	-----	Linear
Step 2	8,000	2.5 hr	-----	Linear
Step 3	8,000	-----	20,000 V-hr	Rapid
Total		5.3 hr	~30,000 V-hr	
17 cm	Voltage	Time	Volt-Hours	Ramp
Step 1	250	20 min	-----	Linear
Step 2	10,000	2.5 hr	-----	Linear
Step 3	10,000	-----	40,000 V-hr	Rapid
Total		7 hr	~50,000 V-hr	

4.4 Completion of IEF.

1. When the electrophoresis run has been completed, remove the IPG strips from the focusing tray and transfer them gel side up into a new or clean, dry disposable rehydration/equilibration tray which matches the length of the IPG. Hold the strips vertically with the forceps and let the mineral oil drain from the strip for ~5 seconds before transfer. Maintain the IPG strips in the same order as in the focusing tray.

After IEF it is sometimes helpful to remove a subset of the IPG strips and stain them with either IEF stain or Bio-Safe Coomassie stain to assess the quality of the focusing step. A procedure for staining IPG strips after the IEF step is described in section 4.5. Staining two of the six strips focused is recommended. However, this IEF stain step is optional.

For 7 cm or 11 cm strips, after completion of the focusing step it is recommended to remove two of the focused strips and stain them following section 4.5, steps 1-4. During the staining step (section 4.5, step 4), a 1 hr incubation in staining solution, it is recommended to equilibrate and mount the remaining strips on the second-dimension gels (section 4.6). Once the SDS-PAGE gels are running, return to section 4.5, step 5 to destain the first-dimension stain.

For 17 cm strips, after the completion of the focusing step it is recommended to remove two of the focused strips and stain them following section 4.5. The remaining strips (gel side up in the disposable rehydration/equilibration tray per section 4.4, step 1) can be covered with the tray cover, and then wrapped in plastic wrap and placed at -70°C for storage. The second-dimension SDS-PAGE gels can then be set up and run on Day 2.

2. Proceed to section 4.5 to stain two of the strips, and then proceed to section 4.6 with the remaining strips. Alternatively, the tray containing the remaining IPG strips can be covered, wrapped in plastic wrap and placed at -70°C for storage. The second-dimension SDS-PAGE gels can then be run at a later time.

4.5 Staining IPG Strips with IEF Stain or BioSafe Coomassie Stain.

1. Transfer 2 of the IPG strips to a clean, dry piece of blotting filter paper with the gel side facing up.
2. Thoroughly wet a second filter paper of the same size with nanopure water. Carefully lay the wet filter paper onto the IPG strips. Press firmly over the entire length of the strips. Do NOT squish the gels. When finished, carefully “peel” back the top filter paper. This blotting step removes mineral oil on the surface of the IPG, thereby reducing background staining and generally improving the staining of the IPG strips.
3. Transfer the 2 IPG strips to a staining tray containing approximately 50 ml of Bio-Safe Coomassie stain or Bio-Rad’s IEF stain.
4. Place the tray onto a rocking platform or orbital shaker for 1 hour.
5. Destain the IPG strips twice for 10 minutes each. For Bio-Safe stain use 20 mM Tris-HCl, pH 8.8. For IEF stain use destain solution (catalog #161-0438). Complete destaining of the IPG strips may take several hours. Changing the destain solution several times can accelerate this process.

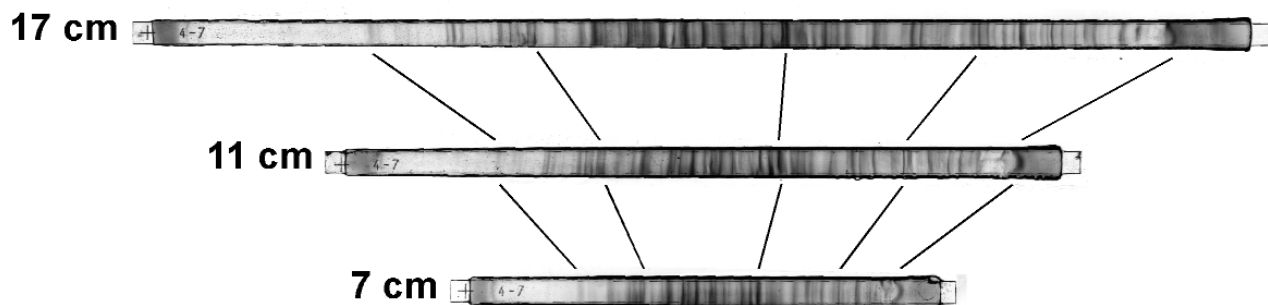


Fig. 9. Isoelectric focusing of *E. coli* protein sample in 7 cm, 11 cm, and 17 cm IPG strips followed by staining with IEF gel staining solution.

- 6 Figure 9 shows the expected pattern when IPG strips of 17 cm, 11 cm, and 7 cm are stained with IEF gel staining solution (catalog # 161-0434) and destained with Coomassie Brilliant Blue R-250 destaining solution (catalog # 161-0438).

4.6 Preparations Needed Before Beginning IPG Equilibration & SDS-PAGE.

Due to time considerations, it is practical to proceed to running the second-dimension SDS-PAGE gel if either the 7 cm or 11 cm IPG strips were used. If 17 cm IPG strips were focused, the length of the SDS-PAGE step is prohibitive in most circumstances to completing this step the same day. In this case, the IPG strips can be frozen at -70°C as described above (section 4.4, step 2) and the SDS-PAGE gel can be run on the next day.

1. Preparation of SDS-PAGE Gels.

- a Remove from the refrigerator the same number of 8-16% precast polyacrylamide gels as the number of IPG strips to be run in the second dimension.
- b Open the packaging for each gel and remove the gels.
- c Remove the IPG comb from each gel and rinse the well briefly with nanopure water using a water bottle.
- d Place the rinsed gels into a tray and when all have been processed, cover the tray with plastic wrap to prevent the gels from drying out.

2. Preparation of 1X SDS-PAGE Gel Running Buffer.

- a Prepare sufficient 1X Tris/glycine/SDS (TGS) running buffer to run the number gels of the size decided upon. See section 5, Appendix, for the recipe for this buffer. Bio-Rad offers a convenient 10X TGS stock solution which is easily diluted with distilled water to make 1X running buffer (See Section 6 for ordering information).

3. Preparation of Equilibration Buffer I and II.

The equilibration buffers should be prepared about 15 minutes before use. If the IPG strips were frozen at -70°C , they can be removed from the freezer and placed onto the lab bench to thaw at this time. The strips require 10-15 minutes to thaw. It is best to not 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.

- a Remove the kit from the refrigerator and unpack the 2 bottles of equilibration buffer I (WHITE or SILVER caps), the 2 bottles of equilibration buffer II (RED caps), the bottle of 30% glycerol (CLEAR cap), the 2 bottles of iodoacetamide (RED caps), and the bottle of overlay agarose (CLEAR cap).
- b Remove the crimp and open the two bottles of equilibration buffer I and equilibration buffer II. (NOTE: if 3 or fewer 17 cm IPGs, 5 or fewer 11 cm IPGs, or 8 or fewer 7 cm IPGs are to be separated in the second dimension, then only 1 bottle each of equilibration buffer I and II and 1 bottle of iodoacetamide is needed.)

Prepare Equilibration Buffer I: To each one bottle of equilibration buffer I carefully add 13.35 ml of the supplied 30% glycerol solution. Each bottle contains a stirbar. Place the bottle onto a stirplate and mix until all the solids have completely dissolved. It may be necessary to periodically swirl the contents of the bottle to dislodge solids remaining on the walls of the glass bottle. Generally, the solids will be dissolved in less than 5 minutes. The bottle will chill as the urea in the solids dissolves. To expedite this buffer reconstitution process the bottle can be warmed slightly in the palm of the hand or placed into a water bath set for 25-30°C as the solution stirs. Do not heat above 30°C.

Prepare Equilibration Buffer II: To each bottle of equilibration buffer II carefully add 13.35 ml of the supplied 30% glycerol solution. Each bottle contains a stirbar. Place the bottle onto a stirplate and mix until all the solids have completely dissolved as described above for equilibration buffer I.

4.7 IPG Equilibrations.

1. If the IPGs were frozen at -70°C then confirm that the strips have thawed before continuing. Frozen IPG strips containing sample are opaque white and turn to clear after thawing and redissolving of the urea present inside each strip.
2. Using Table 3 as a guide, add the indicated volume of equilibration buffer I to each channel containing an IPG strip (gel side up per section 4.4).
3. Place the tray on an orbital shaker and gently shake for 10 minutes. Select a slow shaker speed to prevent the buffer from sloshing out of the tray.
4. After placing the IPG strips to shake in equilibration buffer I, proceed to complete the preparation of equilibration buffer II. Add the contents of one bottle of iodoacetamide to each bottle of equilibration buffer II. Return the bottle(s) to stir until the iodoacetamide is fully dissolved.
5. At the end of the 10 minute 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 rehydration/equilibration tray (left side in Figure 4), until the tray is positioned vertically. Take care not to pour out the liquid too quickly at first as the strips may slide out of the tray. When most of the liquid has been decanted, “flick” the tray a couple times to remove the last few drops of equilibration buffer I.

6. Using Table 3, add the indicated volume of complete equilibration buffer II (containing iodoacetamide) to each strip.
7. Return the tray to the orbital shaker for 10 minutes.
8. During the incubation, melt the overlay agarose solution in a microwave oven using the following method.
 - a Loosen the cap of the bottle of overlay agarose and place the bottle in the center of a microwave oven.
 - b Microwave on high 45 – 60 seconds until the agarose liquifies. Times will vary depending upon the power of the microwave. It is best to stop the microwave after 30 seconds and swirl the bottle to mix the solution. Then, return the bottle and microwave for 15 additional seconds, swirl the liquid again and repeat this again if needed. CAUTION should be taken as the overlay agarose contains SDS, which when heated can cause bubbling over of the agarose solution. Set the overlay agarose solution aside. If desired a stirbar can be added and the bottle set to stir slowly.
9. Discard the equilibration buffer II by decanting at the end of the incubation period as described in step 5 above.

Table 3.

Strip Length	7 cm	11 cm	17 cm
Equilibration Buffer I	2.5 ml	4 ml	6 ml
Equilibration Buffer II	2.5 ml	4 ml	6 ml

4.8 SDS-PAGE.

- 1 Fill a 100 ml graduated cylinder or a tube that is the same length or longer than the IPG strip length with 1X Tris-glycine-SDS running buffer. Use a Pasteur pipette to remove any bubbles on the surface of the buffer.
- 2 Finish preparing the SDS-PAGE gels by blotting away any excess water remaining inside the IPG well using Whatman 3MM or similar blotting paper. Lay the gels onto the bench with the top of the gel facing you and the back (tall) plate on the bottom; see Figure 10.

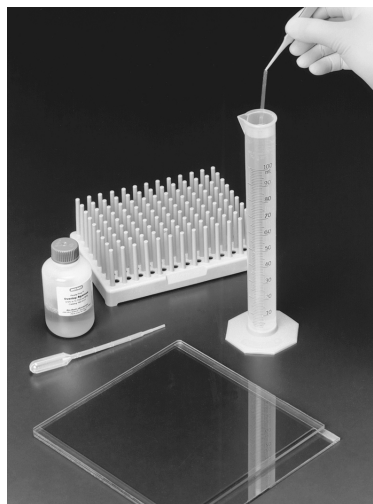


Fig. 10. Rinsing the ReadyStrip in 1 X TGS running buffer.

3. Remove an IPG strip from the disposable rehydration/equilibration tray and dip briefly into the graduated cylinder containing the 1X Tris/glycine/SDS running buffer, as shown in Figure 10. Lay the strip gel side up and onto the back plate of the SDS-PAGE gel above the IPG well (Figure 11). Repeat this process for any remaining IPG strips.

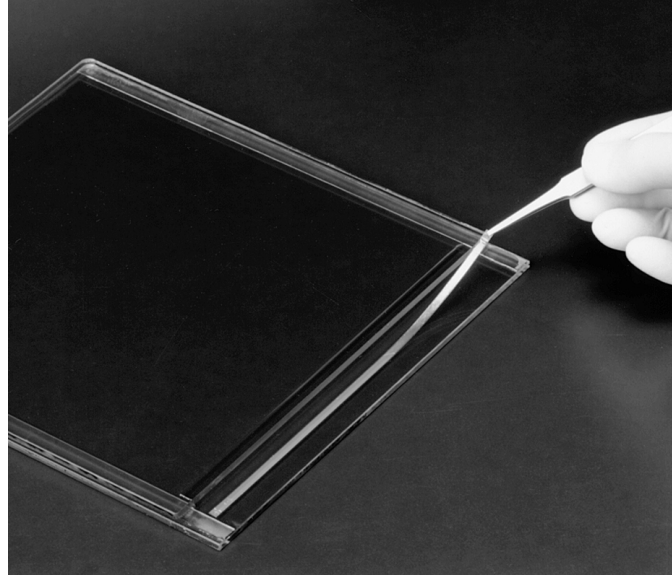


Fig. 11. Placing the ReadyStrip gel side up on the back plate of the SDS-PAGE gel.

4. Take the first SDS-PAGE gel with the IPG strip resting on the back plate and hold or stand vertically with the short plate facing towards you as in Figure 12. Use a pasteur pipette or a Disposable Plastic Transfer Pipet (Bio-Rad catalog # 223-9522) and pipet overlay agarose solution into the IPG well of the gel.



Fig. 12. SDS-PAGE gel with ReadyStrip held vertically in rack. Pipetting overlay agarose into the IPG well.

- Using the forceps, carefully push the strip into the well as shown in Figure 13, taking care not to trap any air bubbles beneath the strip. When pushing the IPG strips with the forceps be certain the forceps are pushing on the plastic backing to the strip and not the gel matrix.

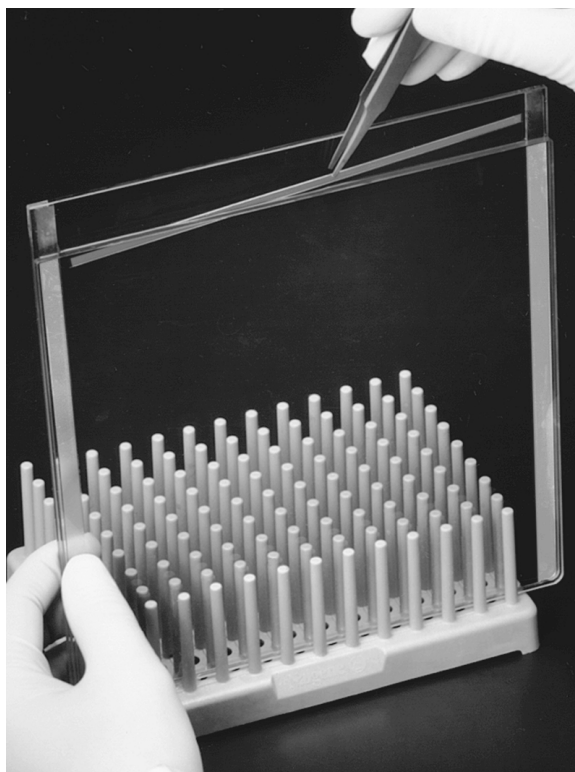


Fig. 13. Sliding ReadyStrip into IPG well filled with melted overlay agarose.

- Stand the gel(s) vertically by placing them in the gel box (Criterion system) or in a test tube rack. Allow the agarose to solidify for 5 minutes before proceeding.
- Mount the gel into the gel box per the instructions provided with the apparatus.
- Fill the reservoirs with 1X Tris/glycine/SDS running buffer and begin the electrophoresis. Table 4 lists the electrophoresis run conditions for each of Bio-Rad's three SDS-PAGE formats. The migration of the Bromophenol Blue, present in the overlay agarose solution, is used to monitor the progress of the electrophoresis.

Table 4.

Strip Length	7 cm	11 cm	17 cm
Electrophoresis cell	Mini-PROTEAN	Criterion	PROTEAN II XL
Conditions	200 V, constant	200 V, constant	16 mA/gel for 30 min, then 24 mA/gel for ~5 hr
Approximate run time	40 min	65 min	5.5 hr

4.9 STAINING.

The 2-D starter kit was designed so that sufficient protein sample loaded in the IEF dimension readily stain second-dimension SDS-PAGE gel with widely available Coomassie Blue stains such as Bio-Rad's Bio-Safe Coomassie stain and Coomassie Brilliant Blue R-250 stain. These staining protocols are rapid and easy to perform so that the 2-D electrophoresis results can be quickly assessed. A description of each staining protocol is provided below.

1. BioSafe Coomassie Stain.

- a Fill enough staining trays with nanopure water for the number of gels run and set aside (for Ready Gel and Criterion gels use 200 ml per gel and for PROTEAN II Ready Gel gels use 400 ml per gel).
- b At the conclusion of the SDS-PAGE, open each gel cassette and place each gel into a tray with water.
- c Wash the gels 3 times for 5 minutes each. Add fresh water for each wash.
- d Add enough Bio-Safe stain to completely cover each gel. For Ready Gel and Criterion gels about 50 ml per gel is sufficient. For PROTEAN II Ready Gel gels use about 100 ml of the stain.
- e Place each gel on a rocker or orbital shaker and shake for at least 60 minutes. The gels can be left in the stain overnight if desired.
- f Discard the stain and wash the gels twice for 15-30 minutes with water. Longer water washes may be needed to remove remaining background. The gels can be stored in water for several days.
- g Figure 14 shows the expected 2-D gel images of the *E. coli* protein sample run on each of the 3 different gel formats (7 cm/Ready Gel, 11 cm/Criterion, and 17 cm/PROTEAN II Ready Gel) and stained with Bio-Safe Coomassie Stain.

2. Coomassie Brilliant Blue R-250 Stain.

- a Add enough Coomassie Brilliant Blue R-250 stain to one or more staining trays to completely cover each gel.
- b At the conclusion of SDS-PAGE, open each gel cassette and place each gel into the stain.
- c Place each gel on a rocker or orbital shaker and shake for at least 60 minutes. Discard the stain when complete.
- d Destain the gels with destain solution (10% acetic acid/40% methanol in water) until the background staining is acceptable. For best results change the destain solution several times. The gels can be stored for several days in a solution of 10% acetic acid.
- e Figure 15 shows the expected 2-D gel images of the *E. coli* protein sample run on each of the 3 different gel formats (7 cm/Ready Gel, 11 cm/Criterion, and 17 cm/PROTEAN II Ready Gel) and stained with Coomassie Brilliant Blue R-250 stain.

4.10 Imaging.

- 1 To preserve the gel images, the destained gels can be imaged on a densitometer such as Bio-Rad's GS-800 calibrated imaging densitometer (catalog # 170-7980). Alternatively, gels can be equilibrated for at least 30 minutes in gel drying solution (catalog # 161-0752) and dried.

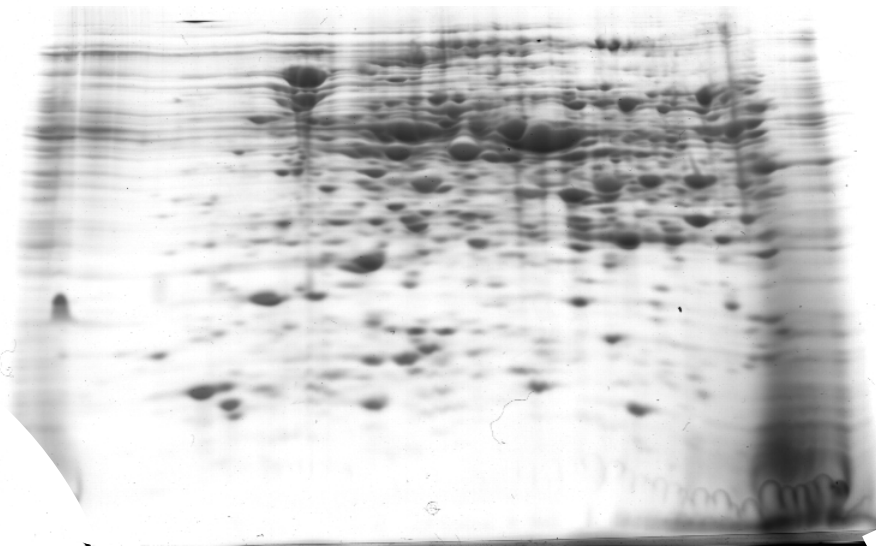


Fig. 14 A. Ready Gel stained with Bio-Safe stain.

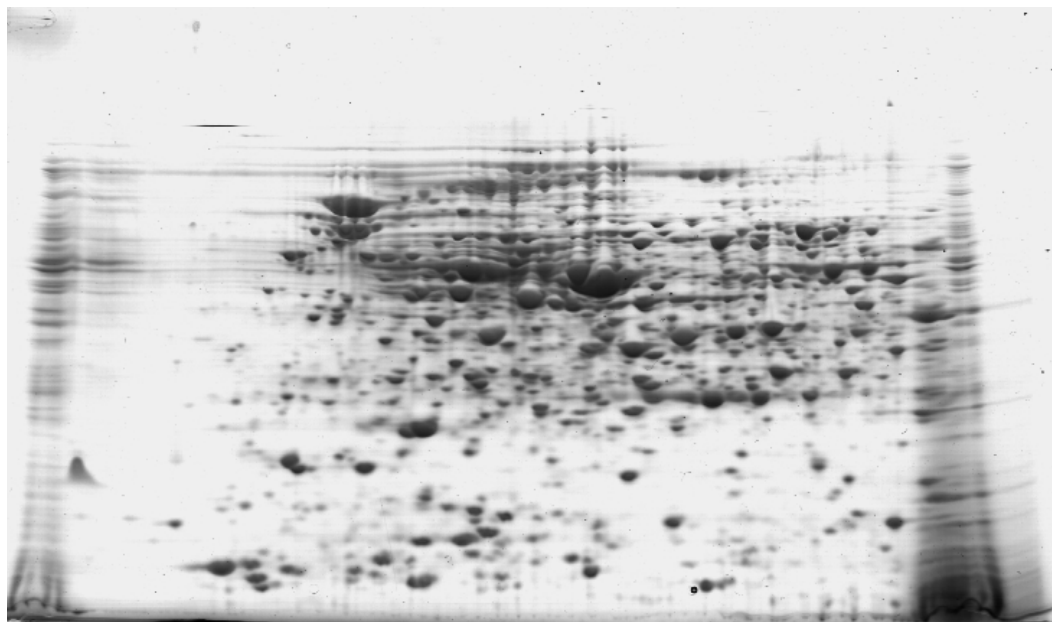


Fig. 14 B. Criterion stained with Bio-Safe stain.

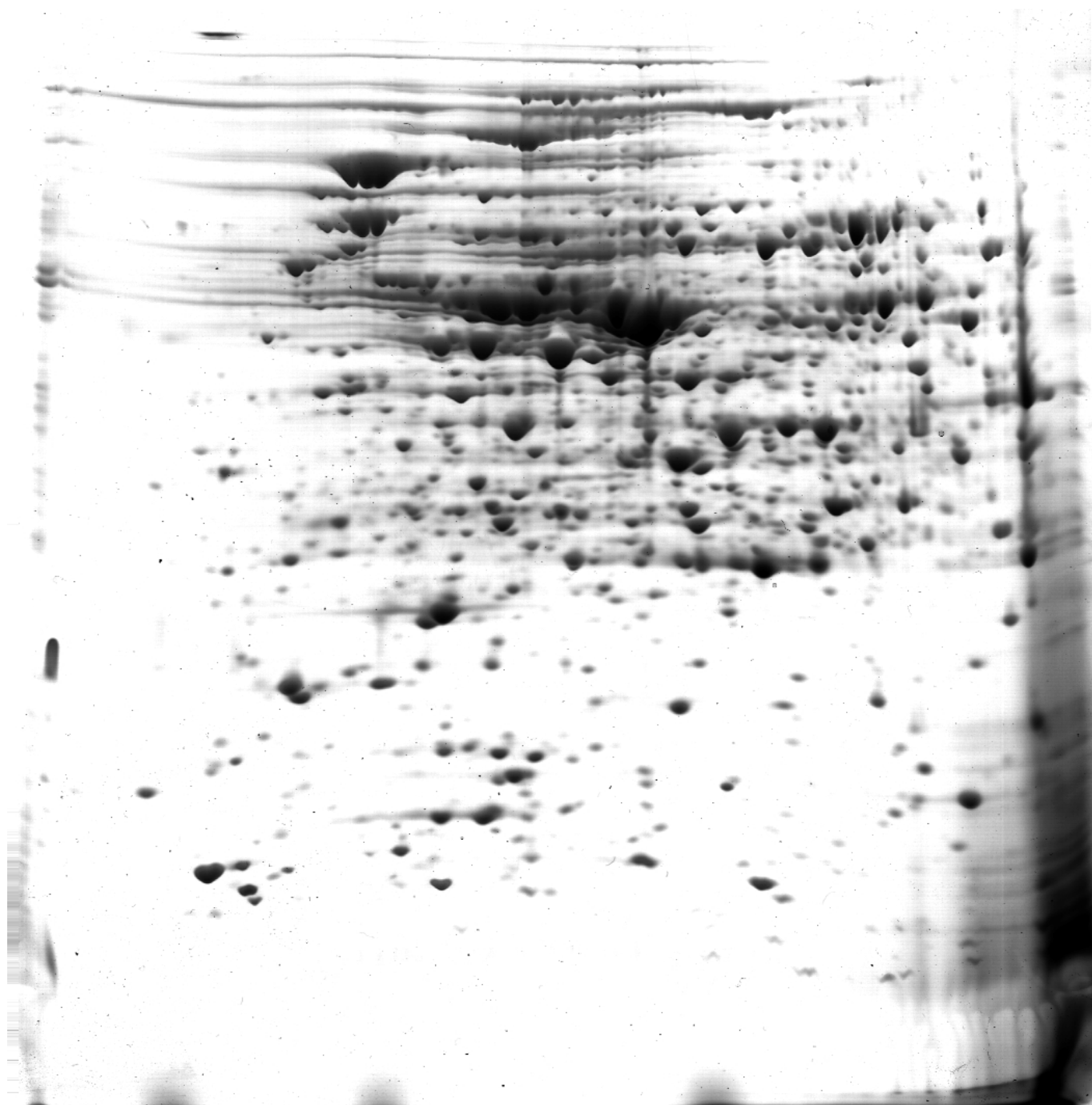


Fig. 14 C. PROTEAN Ready Gel stained with Bio-Safe stain.

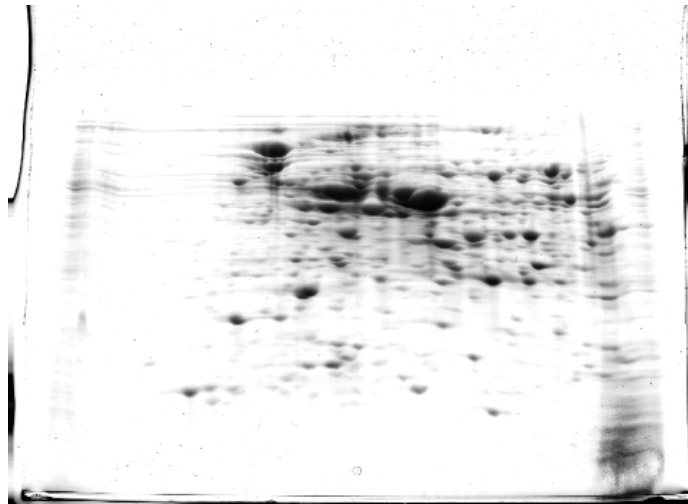


Figure 15 A. Ready Gel stained with Coomassie Blue R-250 stain.

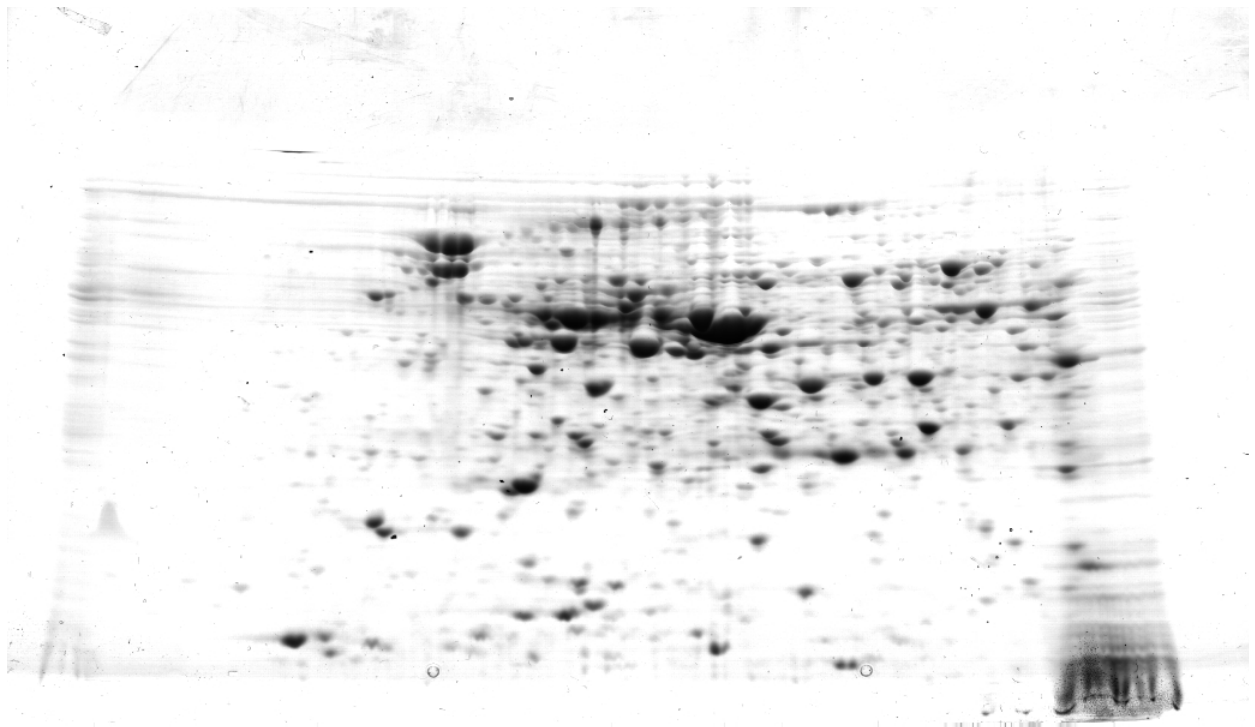


Fig. 15 B. Criterion stained with Coomassie Blue R-250 stain.

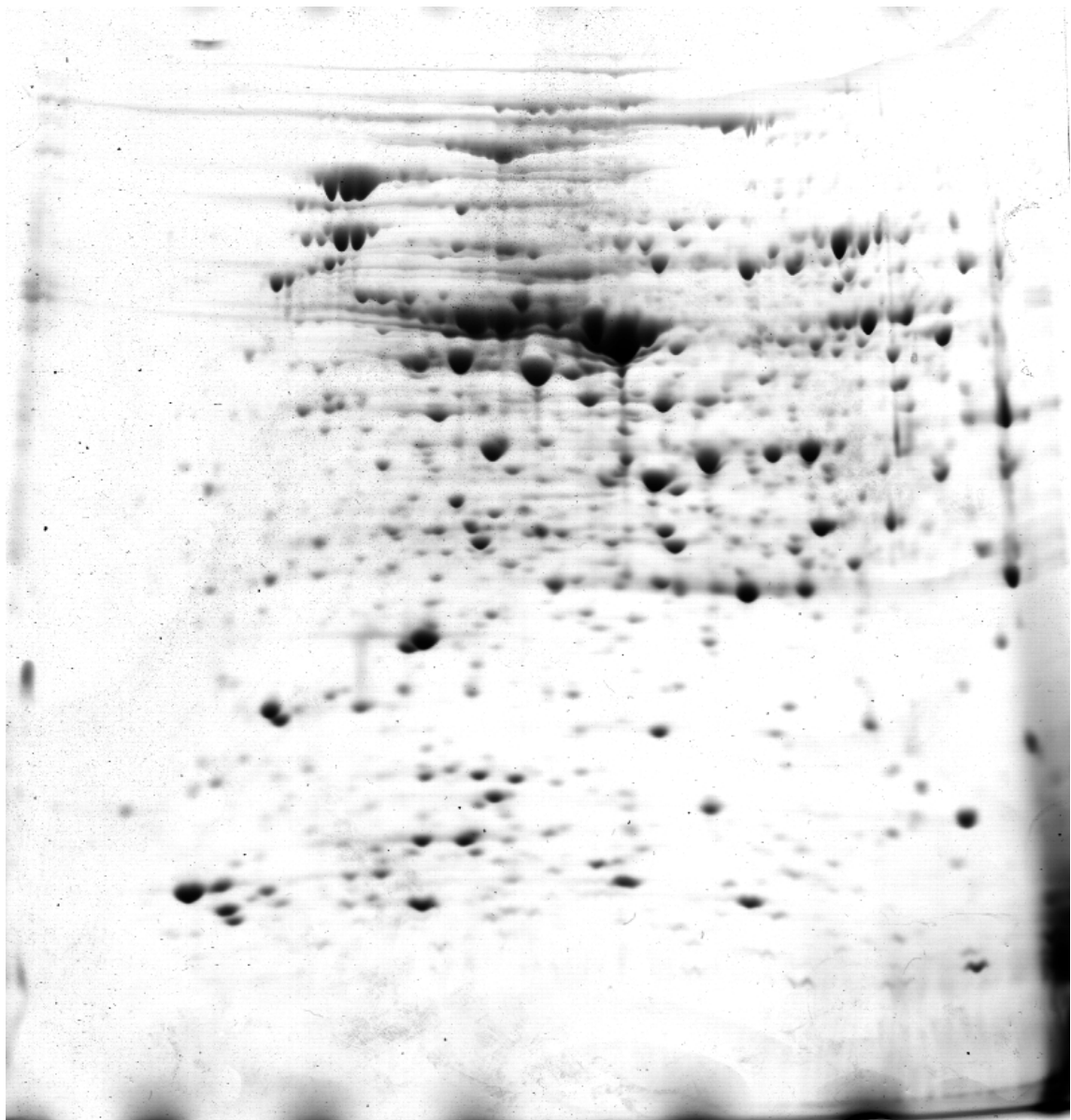


Figure 15 C. PROTEAN Ready Gel stained with Coomassie Blue R-250 stain.

Section 5

Appendix

A Buffer and Reagent Preparation

To avoid introducing experimental error when using this kit, we suggest using premixed buffers and stains. These can be found in section 6, Product Information.

1X Tris/glycine/SDS running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3)

Tris base	3.03 gm
Glycine	14.4 gm
SDS	1.0 gm
Water	to 1 L, do not adjust pH

Coomassie Brilliant Blue R-250 stain solution (0.1% Coomassie Blue R-250 in 40% MeOH, 10% HOAc)

Coomassie Blue R-250	1.0 gm
Water	500 ml
Acetic acid	100 ml
Methanol	400 ml

Destain solution (10% acetic acid, 40% methanol)

Water	500 ml
Acetic acid	100 ml
Methanol	400 ml

B Programming the PROTEAN IEF Cell

New method programming instructions are also found in the PROTEAN IEF cell manual.

1. - Select the **NEW METHOD** mode to program a new method

REHYDRATION	>
PRESET METHOD	>
STORED METHOD	>
NEW METHOD	>

2. - Use the alphanumeric keypad to enter a program name.

- Select **NO** for Rehydration
- Use default FOCUS TEMP of 20°C
- Press **NEXT**

NAME: "Ecoli"	>
REHYDRATION	Yes NO >
FOCUS TEMP	20 °C >
	NEXT >

3. - Enter step 1 final voltage of **250 V**

- same voltage for all strip lengths)
- Select LINEAR ramp. Use key next to arrow, >, to move the brackets, [], around the selection
- Press NEXT

S 01	250 >
SELECT VOLTAGE SLOPE	
LINEAR	[↑] >
	NEXT >

4. - The first line displays the step number, slope and voltage
 - Select **HRS:MIN** (default)
 - Enter time of **20** min.
 - Press **NEXT**

S 01	250 V	>
HRS:MIN	vhours	>
ENTER TIME:	00:20	>
	NEXT	>

5. - Select **ADD** (default)
 - Enter Step **2**
 - Press **NEXT**

ADD inset delete	>
ENTER STEP #	2 >
	NEXT >
METHOD COMPLETE	>

6. - Enter step 2 final voltage
 7 cm: **4,000 V**; 11 cm: **8,000 V**; 17 cm: **10,000 V**)
 - Select **LINEAR** ramp. Use key next to arrow, >, to move
 the brackets, [], around the selection
 - Press **NEXT**

S 02	xxxx V	>
SELECT VOLTAGE SLOPE		
LINEAR	[↗]	>
	NEXT	>

7. - The first line displays the step number, slope and voltage
 - Select **HRS:MIN** (default)
 - Enter time
 (7 cm: **2:00**; 11 cm: **2:30**; 17 cm **2:30**)

S 02	xxxx V	>
HRS:MIN	vhours	>
ENTER TIME:	xx:xx	>
	NEXT	>

8. - Select **ADD** (default)
 - Enter step **3**
 - Press **NEXT**

ADD inset delete	>
ENTER STEP #	3 >
	NEXT >
METHOD COMPLETE	>

9. - **Enter** step **3** final voltage
 (7 cm: **4,000 V**; 11 cm: **8,000 V**; 17 cm: **10,000 V**)
 - Select **RAPID** ramp. Use key next to arrow, >, to move
 the brackets, [], around the selection
 - Press **NEXT**

S 03	xxxx V	>
SELECT VOLTAGE SLOPE		
RAPID	[↖]	>
	NEXT	>

10. - The first line displays the step number, slope and
 voltage
 - Select **VHOURS** (default)
 - Enter **time** (volt hours)
 (7 cm: **10,000**; 11 cm: **20,000**; 17 cm **40,000**)
 - Press **NEXT**

S 03	xxxx V	>
HRS:MIN	VHOURS	>
ENTER TIME:	xxxxx	>
	NEXT	>

11. - Select **METHOD COMPLETE**

ADD inset delete	>
ENTER STEP #	>
NEXT	>
METHOD COMPLETE	>

12. - The save method screen is displayed when the name entered is displayed

- Select **YES**
- Press **NEXT**

"Ecoli"	>
SAVE METHOD? YES no	>
NEXT	>

13. - Enter number of gels

- Press  to start

ENTER # OF GELS:	#	>
μA LIMIT/GEL	50	>
FOCUS TEMP	20 °C	>
PRESS  TO START		>

Section 6

Product Information

Catalog Number	Product Description
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2-D Starter Kit accessories

163-2105	ReadyPrep 2-D Starter Kit
163-2106	ReadyPrep Rehydration/Sample Buffer, 1 bottle, 10 ml
163-2107	ReadyPrep Equilibration Buffer I, 1 bottle, 20 ml
163-2108	ReadyPrep Equilibration Buffer II, 1 bottle, 20 ml
163-2109	Iodoacetamide, 30 gm
163-2110	ReadyPrep <i>E. coli</i> Protein Sample, 1 vial, 2.7 mg
163-2111	ReadyPrep Overlay Agarose, 1 bottle, 50 ml

ReadyStrip IPG Strips

163-2001	ReadyStrip IPG Strips, 7 cm, pH 4-7, 12
163-2015	ReadyStrip IPG Strips, 11 cm, pH 4-7, 12
163-2008	ReadyStrip IPG Strips, 17 cm, pH 4-7, 12

Precast SDS-PAGE gels

161-1394	Ready Gel Precast Gel, 8-16% Tris-HCl, IPG comb, 1
345-0041	Criterion 8-16% Tris-HCl Gel, IPG comb, 1
161-1453	PROTEAN II Ready Gel precast gel, 8-16% Tris-HCl, IPG comb, 1

IEF accessories

163-2129	Mineral Oil
165-4035	Disposable Rehydration/Equilibration Tray with Lid, 7 cm, 25
165-4025	Disposable Rehydration/Equilibration Tray with Lid, 11 cm, 25
165-4015	Disposable Rehydration/Equilibration Tray with Lid, 17 cm, 25
165-4071	Electrode Wicks, precut, 500
165-4070	Forceps, 1
165-4072	Cleaning Brush, 2

Buffers and Stains

161-0732	10X Tris/Glycine/SDS, 1 L
161-0772	10X Tris/Glycine/SDS, 5 L
161-0798	1.5 M Tris-HCl, pH 8.8, 1 L
161-0434	IEF Gel Staining Solution, 1 L *
161-0786	Bio-Safe Coomassie Stain, 1 L
161-0787	Bio-Safe Coomassie Stain, 5 L
161-0436	Coomassie Brilliant Blue R-250 Staining Solution, 1 L *
161-0438	Coomassie Brilliant Blue R-250 Destaining Solution, 1 L

* Requires 161-0438, Coomassie Brilliant Blue R-250 Destaining Solution

