USER'S MANUAL

Investigator™

IEF Tube Gel
Casting and Running Systems

GENOMIC SOLUTIONS™

FUELING INNOVATION.
DRIVING DISCOVERY.™

Printed in the United States of America.

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Throughout this procedure, you will be using high voltage.
Follow instructions in the power supply manual to ensure safe operation.

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Telephone (toll free): 1.877.GENOMIC (436.6642)
Fax: 1.734.975.4808

If you are in Japan, call:
Telephone: +813.3280.0990
Fax: +813.3280.0991

For other locations, contact your local distributor or contact Genomic Solutions by e-mail: technicalsupport@genomicsolutions.com

On the web: www.genomicsolutions.com

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Good luck with your research and do not hesitate to contact us should you have any questions.

OVERVIEW

IEF Tube Gel Casting System

The following components are included with the analytical IEF Tube Gel Casting System:

- Analytical casting funnel
- IEF casting cylinder
- Analytical snap-cap vials (20 per pack)
- Analytical threaded tubes, 26 cm (18 per pack)

Inside each tube there is a specially treated thread. This thread supports the gel after it is extruded to reduce stretching and breaking. The threaded tubes are supplied in three bundles of six tubes and are held together with shrink-wrap.

Additional items are needed for casting preparative gels (page 32).

Store the snap-cap vials in a plastic bag to minimize dust contamination.

Precautions

- Do not cut the shrink-wrap before analytical casting.
- To prevent protein contamination, wear gloves when handling the tubes.
- Do not remove the tubes from the packaging until just prior to use.

Follow the steps below to prepare the IEF casting equipment for use.

1. Rinse the funnel and cylinder in 18 MOhm water to remove any packaging dust. Make sure the hole on each side of the funnel is not obstructed in order to allow water to pass through it during casting.

2. Rinse the snap-cap vials with 18 MOhm water.

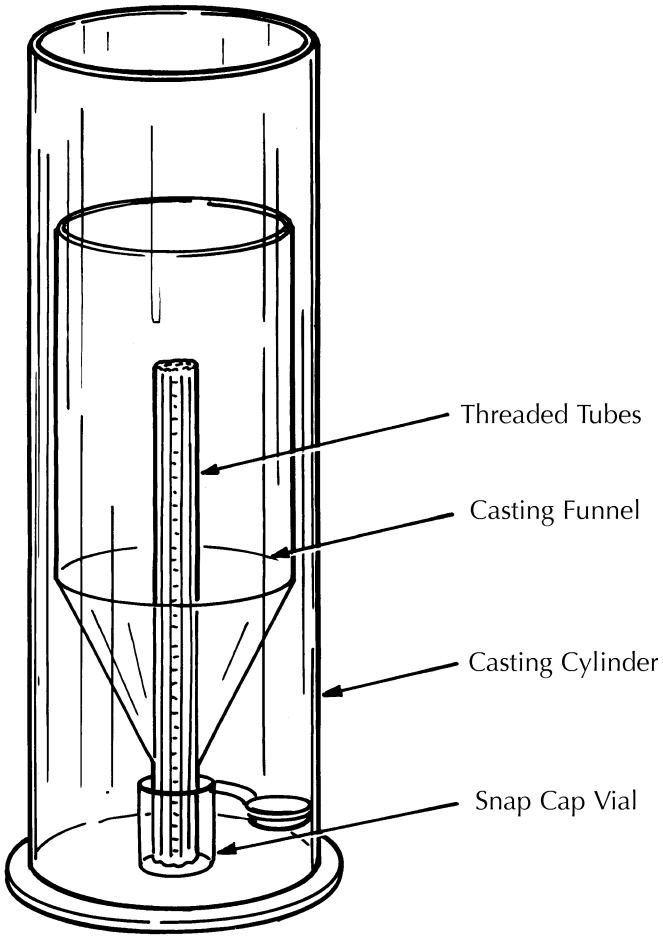

Figure 1. Tube Gel Casting System

ANALYTICAL

Using the IEF Tube Gel Casting System

A maximum of 18 1 mm tube gels can be cast at one time. The IEF Tube Gel Casting System uses water to displace the acrylamide/ampholyte solution into the threaded glass tubes. This procedure results in gels of identical length. For extra convenience, Genomic Solutions provides pre-cast IEF gels (page 32). To cast your own gels, use the following procedures.

Preparing the Threaded Tubes

Threaded tubes for analytical gels are packaged in three bundles of six per pack. The bundles consist of three threaded tube pairs held together with shrink-wrap.

For a run of 15 tubes, you should cast 18 tubes to allow for breakage. You may cast as few as six or as many as 18 gels. Use 6 ml of casting solution even when casting only one bundle of six tubes.

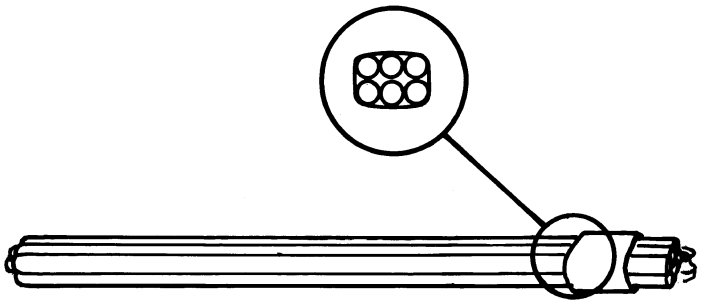

Figure 2. Capillary tube bundle with shrink-wrap

Casting the 1-D Gels

1. Thaw the gel casting solution. DO NOT WARM SOLUTION TO OVER 30°C. THIS CAN CAUSE THE FORMATION OF CYANATE IONS AND LEAD TO PROTEIN CARBAMYLATION. Mix the thawed solution gently to re-dissolve the ingredients.

2. Fill the gel casting cylinder with 1100 ml of 18 MOhm water.

3. To the thawed gel casting solution, add 360 µl of the desired carrier ampholyte and 40 µl of 10% (w/v) freshly prepared ammonium persulfate. (TEMED is not required for polymerization due to the addition of carrier ampholytes.) A few grains of bromphenol blue can be added to the gel casting solution to enhance visualization of the gel surface and monitoring of the casting process.

4. Place one to three bundles of six threaded glass capillary tubes with the shrink wrap down into the vial. Tilt the vial gently to one side as the tubes are placed into the solution to avoid the formation of air bubbles.

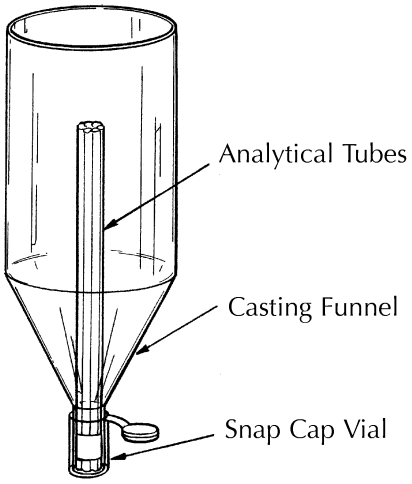

Figure 3. Capillary Tubes Added to Casting Funnel and Snap Cap Vial

5. Guide the small end of the 1-D casting funnel over the tops of the tubes and press the snap cap vial onto the assembly, as shown in Figure 3.

6. Place the gel casting funnel into the casting cylinder containing the 1100 ml of 18 MOhm water. Water will slowly enter through the lower small hole, displacing the casting solution, and then pass more rapidly through the larger hole as the fluid level rises. To prevent uneven filling of the tubes, do not apply any pressure to the funnel when introducing it into the casting cylinder; simply allow it to sink on its own (Figure 4).

7. The gel solution will polymerize within one hour, but to ensure that all acrylamide has polymerized, continue to hold at room temperature for a minimum of two hours.

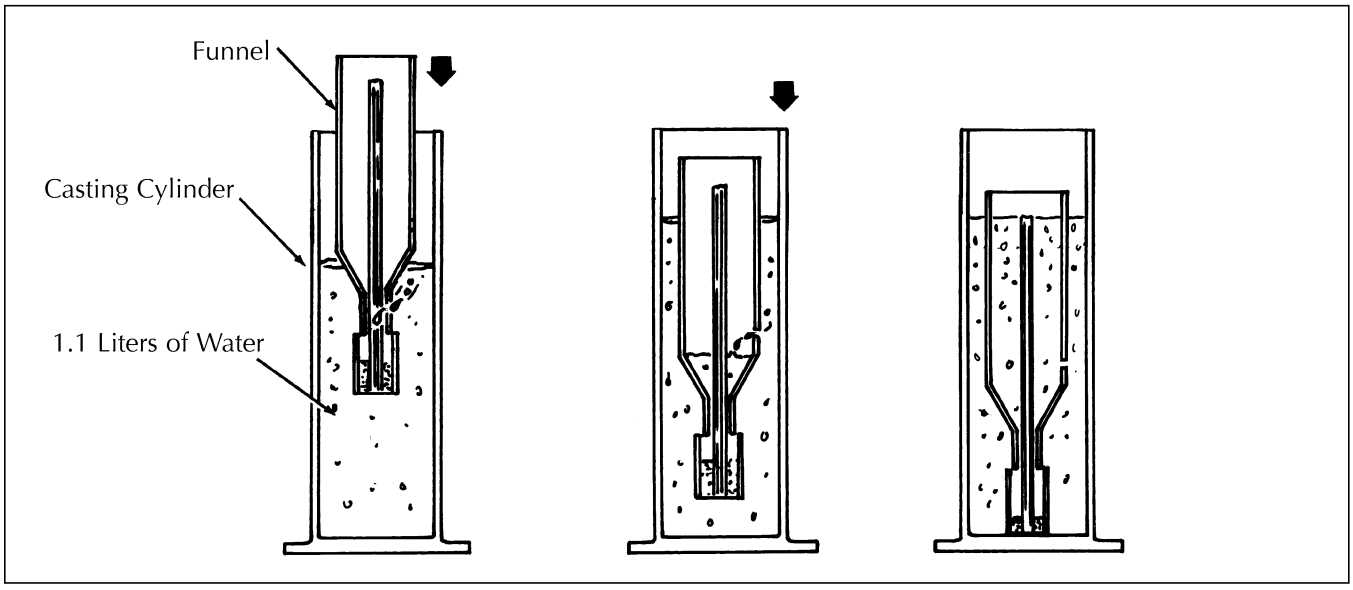

Figure 4. Inserting the Casting Funnel Assembly

8. To remove the tube gels, lift the casting funnel from the casting cylinder. The water will drain out through the two holes in the cylinder. It is convenient to remove the funnel at the sink to avoid spraying water over the work area.

9. Detach the snap-cap vial from the bottom of the casting cylinder and gently pull the bundled tubes from the vial (Figure 5).

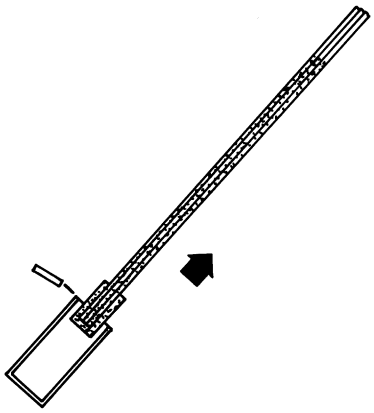

Figure 5.

10. Carefully remove the excess casting solution from the bottom of the tube.

11. The tube bundles are held together with shrink-wrap at the bottom end and are connected in pairs with nylon thread. Cut away the shrink-wrap with a scalpel blade (Figure 6a). Care must be taken to not cut the threads. Separate tube gel pairs by cutting with a scalpel blade or scissors. Care must be taken in order to avoid breaking the tubes. THEY ARE EXTREMELY FRAGILE (Figure 6b).

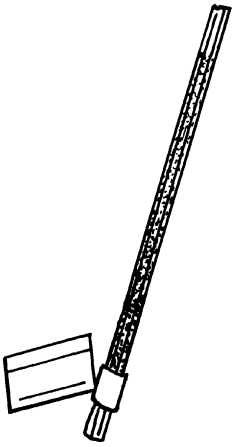

Figure 6a.

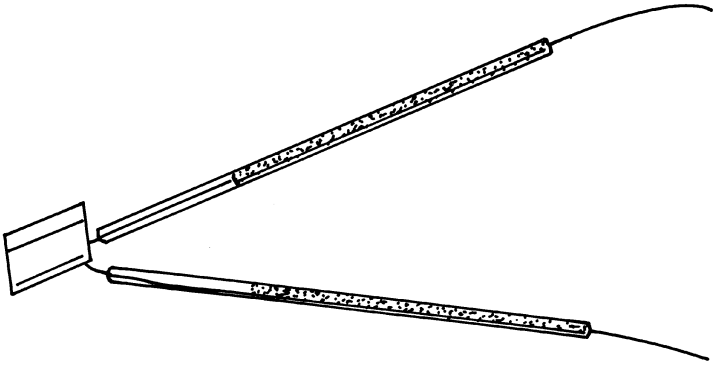

Figure 6b.

12. Gels produce best results if run immediately after casting. However, gels can be stored at 4°C for up to 30 days. Tube gels can be stored in a plastic bag with 1 ml of 18 MOhm water.

USING THE IEF TUBE GEL RUNNING SYSTEM

Fifteen analytical tube gels or eight preparative tube gels can be run at one time. This chapter describes the procedures for running analytical gels. The analytical tube slots are numbered. Each tube can hold a maximum sample volume of 50 µl.

The IEF Tube Gel Running System includes:

- Running tank—0080-0034
- Cover with cable—0080-0033
- Running rack—0080-0035
- Plugs—0080-0038 and 0080-0036
- Grommets—0080-0039 and 0080-0037
- Syringes—0080-0046 and 0080-0044
- Extrusion adapters and trays—0080-0040, 0080-0041 and 0080-0045
- Gel installer tool—0080-0042

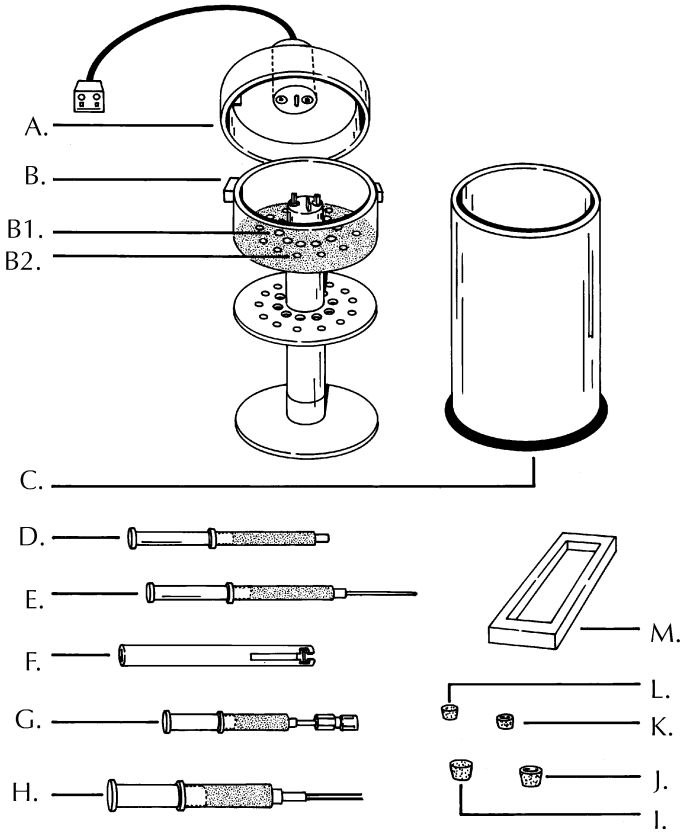

Figure 7. IEF Tube Gel Running System Components

A. Cover and cable—0080-0033
B. Running rack—0080-0035
   B1. Preparative slots
   B2. Analytical slots
C. Running tank—0080-0034
D. Hamilton syringe without needle—0080-0046
E. Hamilton syringe with needle—0080-0046 and 0080-0044
F. Tube gel installer tool—0080-0042
G. Analytical extrusion syringe (with adapter)—0080-0040 and 0080-0041
H. Preparative extrusion (syringe)—0080-0225 (not included with system)
I. Preparative plug—0080-0038
J. Preparative grommet—0080-0039
K. Analytical grommet—0080-0037
L. Analytical plug—0080-0036
M. Equilibration tray—0080-0045

Preparing the IEF Tube Gel Running System

Assembling the system

1. Prepare the anode (lower buffer) and cathode (upper buffer) solutions (page 25).

2. Pour 2L of anode solution into the running tank. Up to 4.5 liters of buffer can be used in the lower reservoir to increase the thermal capacity of the system.

3. Soak the grommets and plugs in 18 MOhm water to remove any sodium hydroxide remaining from previous runs. The water will also act as a lubricant when inserting the tube gels into the grommets.

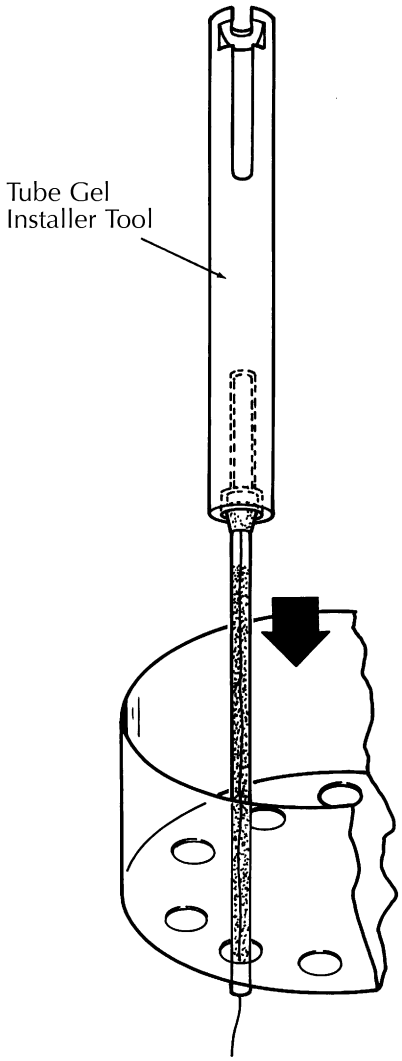

Figure 9. Inserting the tubes into the upper reservoir

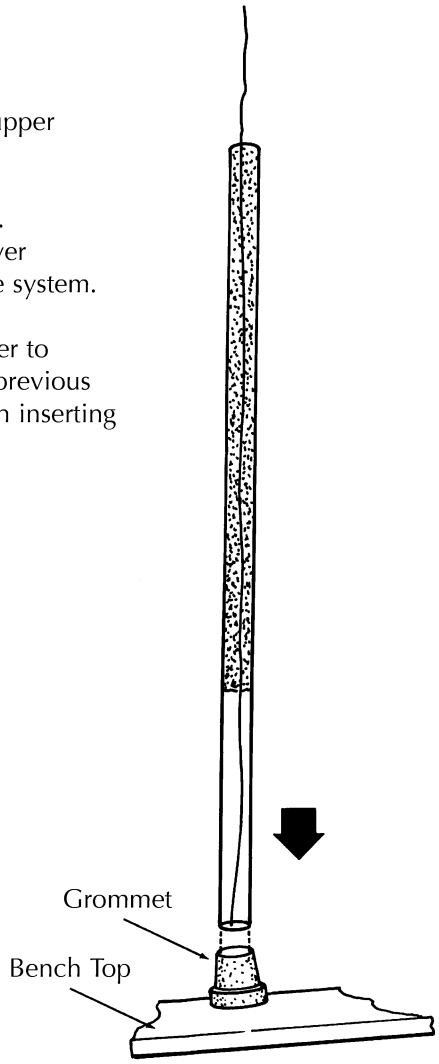

Figure 8a.

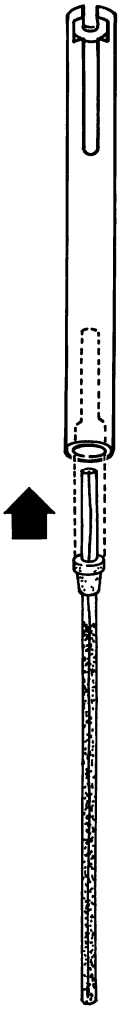

Figure 8b.

4. Place a grommet upside down on the bench top. Insert the top of a tube gel into the grommet and gently push the tube into the grommet (Figure 8a).

5. Once the tube has been inserted into the grommet, you may use the tube gel installer tool to adjust the grommet to its specified position on the tube (Figure 8b).

6. Transfer the tube to the running rack using the gel installer tool. Push down the grommet until its top is snug against the top of the running rack (Figure 9).

7. Continue this process until all tubes are in place. The remaining holes must be blocked by the addition of white plugs for the narrow (analytical) holes, and blue plugs for the large (preparative) holes.

8. Using the Hamilton syringe and needle, fill the well of each tube with upper (cathode) buffer solution.

9. Pour a little upper buffer into the gel rack to verify that there are no leaks.

10. Lower the running rack into the anode solution.

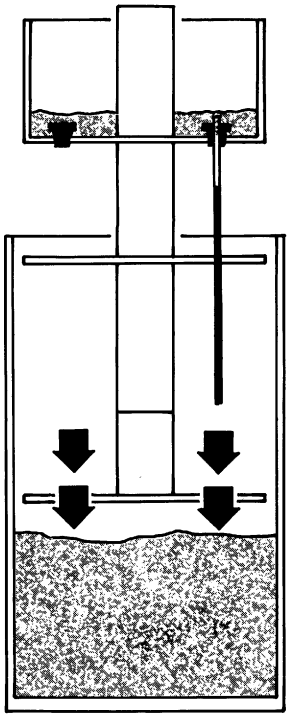

Figure 10. Lower the IEF Running Rack into the Anode Solution

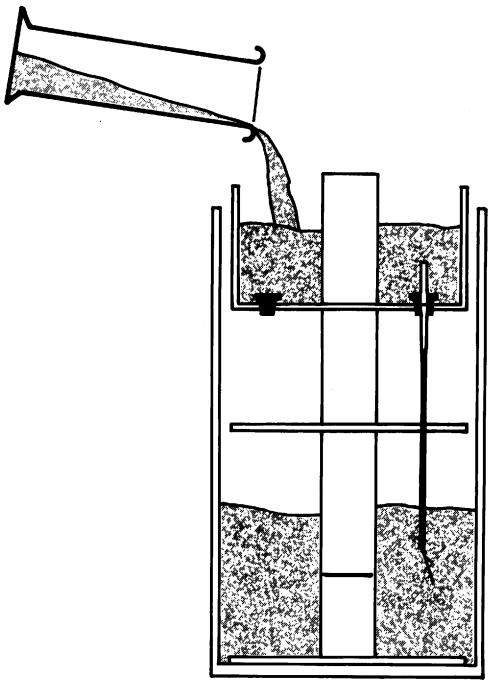

Figure 11. Filling the Upper Reservoir with Cathode Solution

11. Add the remainder of the cathode solution to a total of 800 ml.

12. If cathode solution has not been placed on top of the gels prior to installation (step 8), air bubbles will be trapped on the top of each gel. To remove the bubbles, fill a Hamilton syringe with cathode solution and attach the fine needle supplied with the system. Insert the needle to just above the surface of the gel, and fill the space with solution while slowly withdrawing the needle. This will displace the air bubbles.

13. If using prefocusing step, place lid on cylinder. It will only fit in the correct position. If not prefocusing, proceed to "Loading the Samples", page 16.

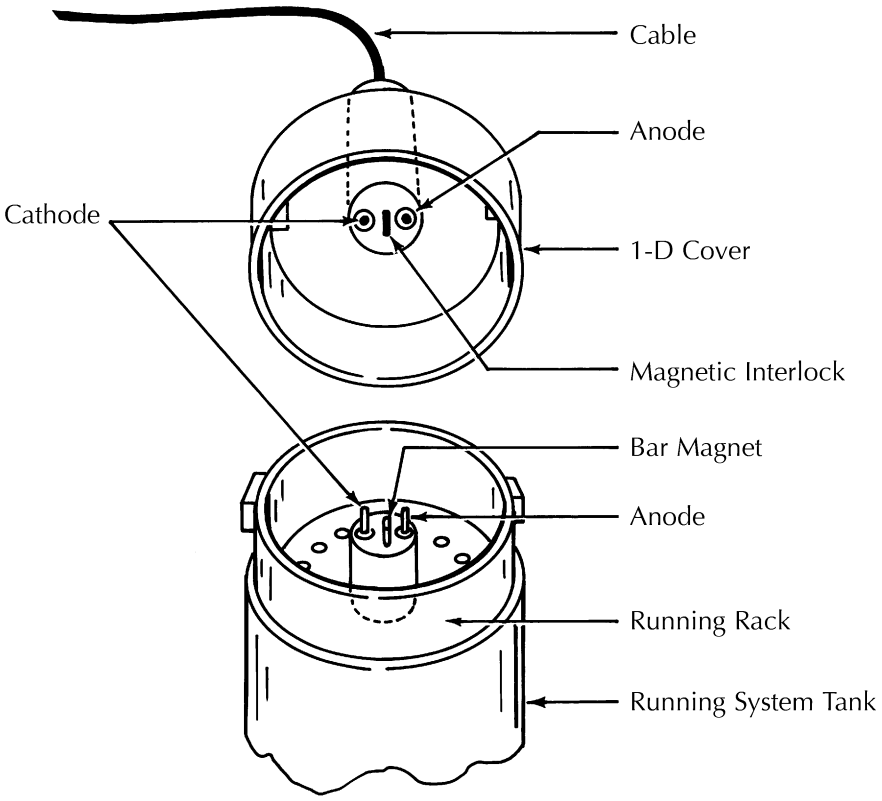

Figure 12. Installing the Cover

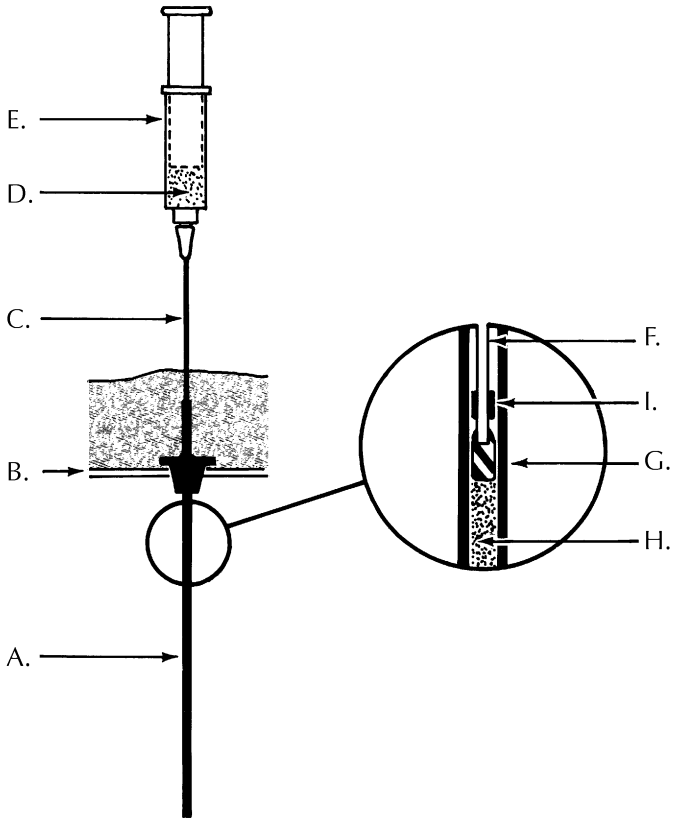

Figure 13. Inserting the Needle into the IEF Tube

A. 1-D tube
B. Rack
C. 3.9-inch (10 cm) needle
D. Sample
E. Hamilton syringe
F. Needle
G. Loaded sample
H. Gel
I. Grommet

Pre-focusing the Gels

NOTE: Experiments in the R & D laboratory indicate that eliminating the pre-focusing step in both analytical and preparative gels can result in improved pattern resolution. If you wish to omit the pre-focusing step, proceed to the section entitled "Loading the Sample."

Programming the Power Supply

Action	Display
Press FUNCTION SETUP	PROCESS (1P, 2F, 3S, 4B)
Press 1 to select pre-focusing.	# OF GELS:xx
Enter the number of 1-D gels you are running in the experiment (1-15). (Example: Press 15/ENTER)	MAX VOLTAGE (V): xxxx
Press 1500 ENTER. For analytical gels, the maximum voltage achieved during pre-focusing should be 1500 volts.	DURATION (h 0-99) xx
Press 2 ENTER to specify two hours for the Duration of the pre-focusing process.	DURATION (m 0-59) xx
Press 30 ENTER to specify 30 minutes. Pre-focusing is complete when the voltage reaches maximum. This should occur within 90-120 minutes. The entered time limit for pre-focusing is 2.5 hours. If maximum voltage is not reached within this time limit, pre-focusing has not occurred.	MAX CURRENT (µA)
Press 110 ENTER to set the maximum current per gel to 110 µA.	

Checking your program

Action	Display
Press FUNCTION SETUP	PROCESS (1P, 2F, 3S, 4B)
Press 1 to select prefocusing.	
Press ENTER to check your previous entry in the prefocus program, starting with the # of GELS. The previously entered value will show on the first line of the screen.	
Press ENTER again to check the MAXIMUM VOLTAGE.	
Repeat for DURATION and MAXIMUM CURRENT.	

Starting the Prefocus Run

Action	Display
Press START.	PROCESS (1P, 2F, 3S, 4B)
Press 1 for prefocusing. Since the power supply can run two processes at once, you have to specify which process is being initiated. Panel lamp 1 will go on. The prefocus review screen will show on the display.	

Monitoring the Prefocus Run

Note: If you are running one process, the review screen for that process remains on the display screen. If you are running more than one process at a time, and you need to monitor the other process, follow the steps below to monitor the process not currently shown on the screen.

Action	Display
Press REVIEW.	PROCESS (1P, 2F, 3S, 4B)
Press 1.	The actual readings for prefocusing are displayed and will be updated once per second throughout the run.
Press ENTER to leave the REVIEW mode.	The time of day.

Finishing the Run

Note: When prefocusing is complete, an alarm will sound to indicate that the maximum voltage has been reached and that the power has been automatically shut off.

Caution: If the alarm sounds and maximum voltage has not ben reached, an error in prefocusing may have occurred. Press REVIEW after the process is complete to check prefocusing.

Action	Display
Press "." to stop beeping.	
Press STOP.	PROCESS (1P, 2F, 3S, 4B)
Press 1 to stop prefocusing.	

Note: If the power fails when a process is running, the supply will restart when power is restored. The screen will display:
!!POWER OUTAGE!! @XX:XX for YY:YY:YY

The variable "XX:XX" is the time of day the power failed, and the "YY:YY:YY" is the total period for which power was out. This screen can be cleared by pressing the ENTER key.

Loading the Samples

Note: At the end of pre-focusing, the power supply will beep. Press ".", "STOP", then "1" "ENTER".
It is now safe to remove the lid and add the sample.

1. Once all bubbles have been removed, samples can be loaded into the wells. A maximum of 50 µl can be loaded on each gel.

2. To load samples, draw each into a 50 µl syringe equipped with a 5 cm fixed needle.

3. Insert the needle into the top of the analytical tube, but do not touch the top of the tube gel.

4. Carefully expel the sample onto the top of the tube gel while slowly withdrawing the needle.

5. Repeat steps 2 through 4 until all of the samples are loaded.

6. When all samples have been loaded, place the lid on the tank. Note that the lid will only fit in the correct position.
7. Turn on the power supply using the rocker switch near the electrical cord inlet at the rear of the unit.

8. The power cable from the running tank lid should now be connected to the power supply socket allocated for iso-electric focusing.

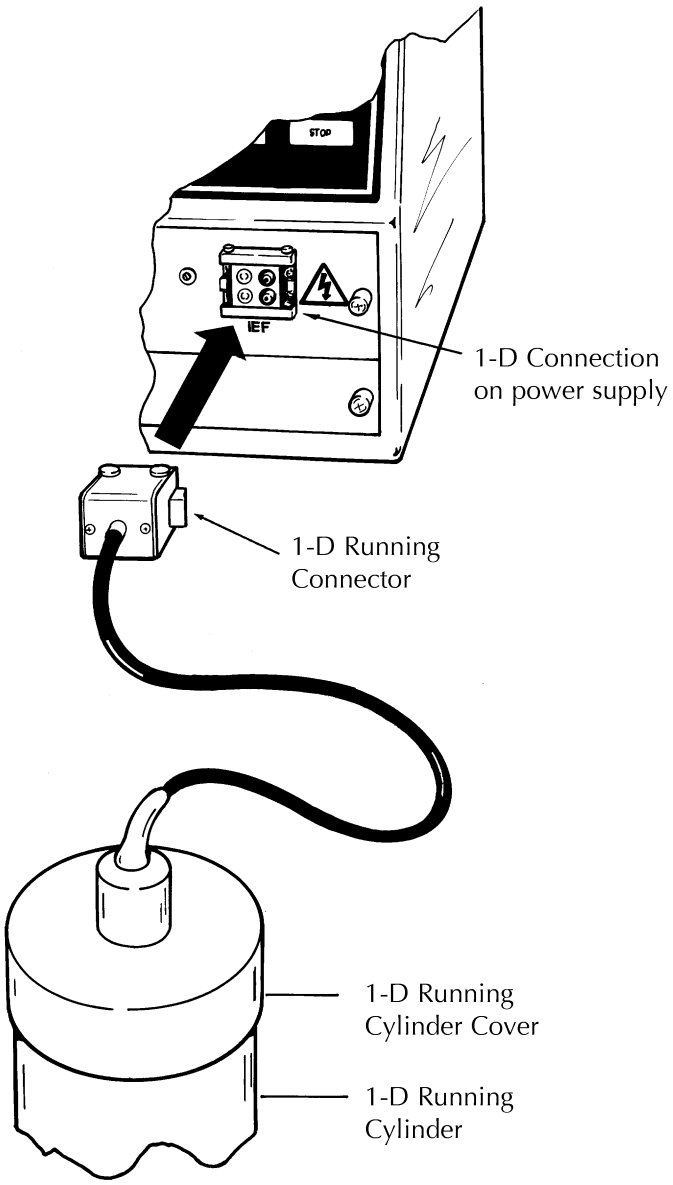

Figure 14. Power Supply Connections

Focusing the Gels

Action	Display
Press FUNCTION SETUP.	PROCESS (1P, 2F, 3S, 4B)
Press 2.	# OF GELS: xx
Enter the number of first dimension gels you are running in the experiment. Example: 15 ENTER.	MAXIMUM VOLTAGE (V): XXXX
Press 2000 ENTER to set the maximum voltage for analytical gels to 2000 Volts.	Vhold (holding voltage): XXX
Press 125 ENTER to set the holding voltage to 125.	DURATION (h 0-99): XX
Enter the number of hours for the duration of your run. 17 hours are recommended for normal operation. Example: Press 17 ENTER.	DURATION m (0-59): XX
Enter the number of minutes (in addition to the hours you already entered) for the duration of your run. 30 minutes are recommended. Example: Press 30 ENTER.	MAX CURRENT (µa): XXX
Press 110 ENTER to set the current per gel to 100 µA. The power supply multiplies this value by the number of gels to calculate the total current.	VOLT HOURS: XXXX
Press 18000 ENTER to set focusing to 18000 Volt-hours.	

Note: For reproducible patterns, the Volt-hours must be the same from run to run.

Checking your program

Action	Display
Press FUNCTION SET UP.	PROCESS (1P, 2F, 3S, 4B)
Press 2 to select focusing.	
Press ENTER to check your previous entry into the prefocusing program, starting with # OF GELS. The previously entered value will show on the first line of the screen.	
Press ENTER again to check the MAX VOLTAGE.	
Repeat for Vhold, DURATION, MAX CURRENT and VOLT HOURS.	

Starting the Run

Action	Display
Press START then 2.	

Monitoring a Focus Run

Action	Display
Press REVIEW.	PROCESS (1P, 2F, 3S, 4B)
Press 2.	The actual reading for focusing are displayed and will be updated once per second throughout the run.
Press ENTER to leave the REVIEW mode.	The time of day.

Finishing the Run
Note: When focusing is complete, an alarm will sound. This indicates that the designated time has elapsed. The display will read "PRESS (.) to disable beeping sound". Once the beeping sound has been disabled, a holding voltage of 125 V will be maintained.

Caution: If the alarm sounds and final volt-hours have not been reached, an error in programming may have occurred. Press REVIEW after the process is complete to check focusing.

Action	Display
Press "." to stop beeping.	
Press STOP.	PROCESS (1P, 2F, 3S, 4B)
Press 2 to stop focusing.	

Note: If the power fails when a process is running, the supply will restart when power is restored. The screen will display: !!POWER OUTAGE!! @XX:XX for YY:YY:YY

The variable "XX:XX" is the time of day the power failed, and the "YY:YY:YY" is the total period for which power was out. This screen can be cleared by pressing the ENTER key.

PREPARING FOR 2-D ELECTROPHORESIS

Extruding the Gels

1. Disconnect the 1-D running cable system from the power supply.

2. Remove the cover of the 1-D running assembly by pushing down on the tabs which pass through the cover. Push the tabs until the cover slips off the inner rack, then remove.

Caution: Do not lift the cover by the center post.

3. Remove the tubes from the 1-D running system using the grommet installer tool. The cathode solution will drain into the anode solution.

4. IMPORTANT: Lay the tubes on a bed of ice to cool and stiffen the gel for approximately 5-10 minutes. This will make the gels easier to extrude. The tubes can remain on ice for up to an hour.

5. Thaw Equilibration Buffers I and II at room temperature in a 30˚C water bath (pages 27-28).

Figure 15. Inserting the top of the Analytical Tube Gel into the adapter

6. Select a tube and insert the top end into the gel extrusion adapter.

7. Turn the adapter by hand until it is tight. The amber latex seal in the cap secures the glass tube and prevents leakage.

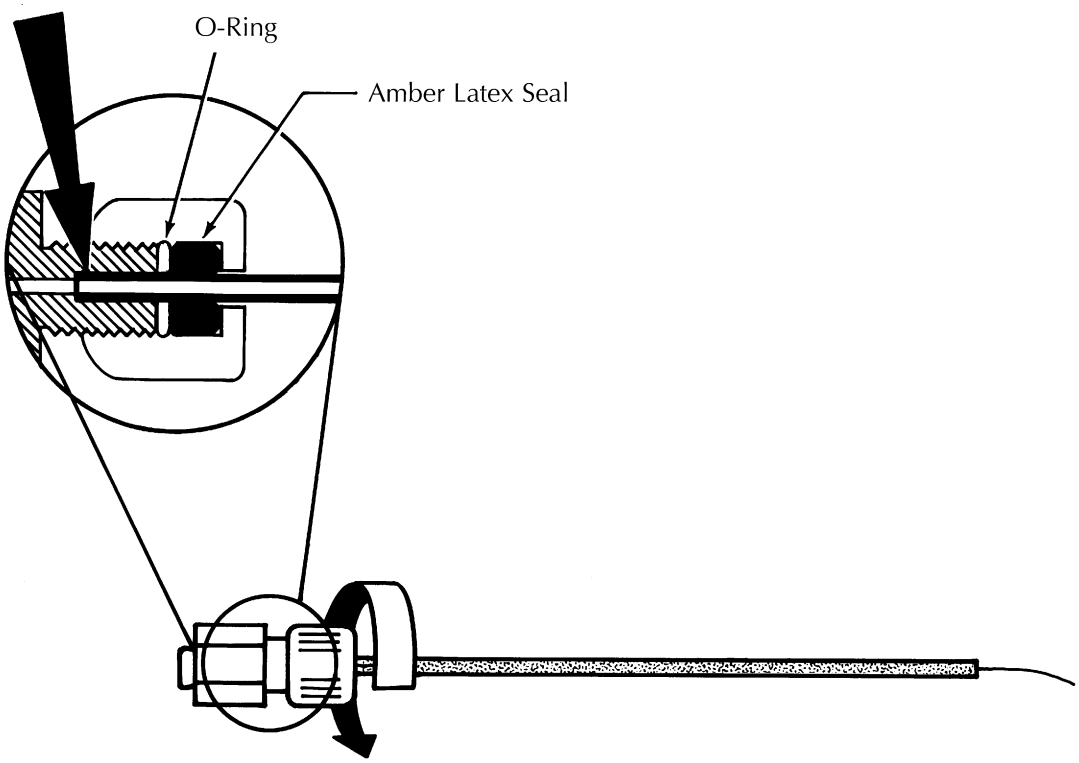

Figure 16. Tightening the Adapter

8. Fill the 1 ml extrusion syringe with 18 MOhm water. Attach the adapter/tube gel assembly to the end of the syringe.

9. Fill an equilibration tray with 5 ml of Equilibration Buffer I. Each tube gel should have its own equilibration tray.

10. Exert constant pressure on the syringe plunger until the acidic end of the gel begins to move out of the tube and is slowly extruded into the equilibration tray.

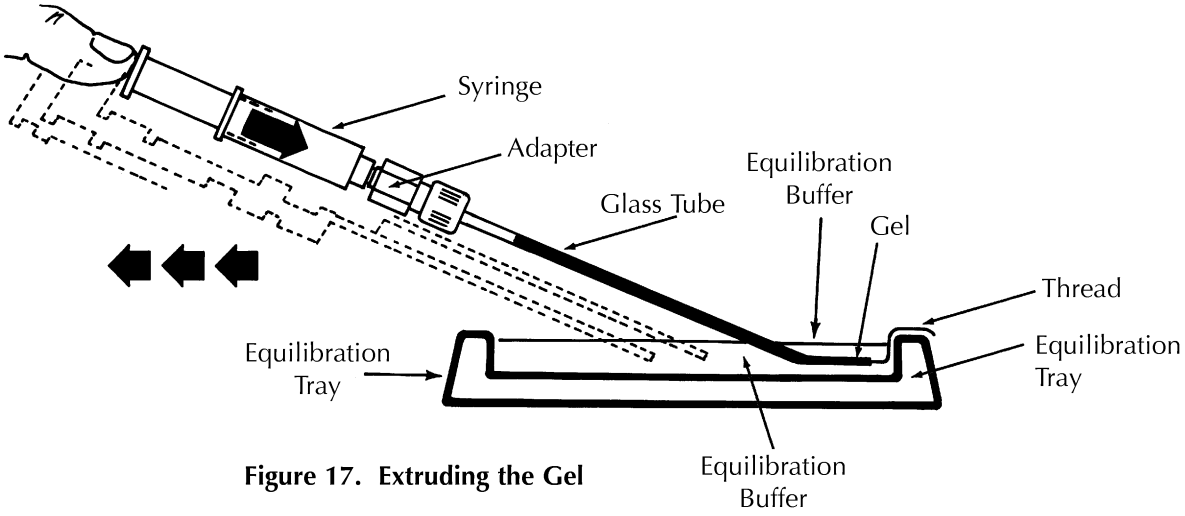

Figure 17. Extruding the Gel

11. Incubate the gel in Equilibration Buffer I for two minutes at room temperature. If you are planning to analyze proteins using mass spectrometry, repeat the equilibration using Equilibration Buffer II in order to alkylate reactive free thiols.

NOTE: Gels are now ready to be loaded on the 2-D gel. Please see 2-D Casting and Running Manual.

PREPARATIVE

USING THE IEF TUBE GEL CASTING SYSTEM

Approximately 10 times more protein can be loaded onto preparative gels than onto analytical gels. This amount of protein is within the range for sequencing using commercially available protein sequencers. See page 32 for Preparative Casting Supplies.

Preparing the Casting Solution

1. Thaw the 1-D Preparative Casting Solution (page 30) at room temperature or in a 30°C water bath.

2. (Optional) De-gas the Solution under vacuum for one to two minutes.

3. Add 78 µl of 10% ammonium persulfate and 25 µl of TEMED to 25 ml of the gel solution, and mix by inversion.

Casting the Preparative Gels

1. Pour 1050 ml of 18 MOhm water into the 1-D preparative casting cylinder. The water temperature should be about 25°C to avoid urea precipitation. Make sure the cylinder base is level.

2. Guide the small end of the preparative funnel into the top of the snap-cap vial and press together tightly.

3. Place up to eight of the two or three mm preparative tubes into the preparative snap-cap vial. Tilt the vial gently to avoid the formation of air bubbles.

4. Slowly lower the snap-cap vial, tubes and funnel combination into the casting cylinder so that the water entering through the lower hole in the funnel overlays the gel solution but does not mix with it.

CAUTION: It is very important to lower the funnel slowly to prevent a rush of water which would mix the water and gel solution.

5. Allow the funnel and vial combination to gradually settle to the bottom of the cylinder.

6. Wait about one hour for the gels to polymerize.

7. Slowly lift the funnel and vial combination from the casting cylinder. Allow the water to drain as you lift.

NOTE: It is convenient to remove the funnel at the sink to avoid spraying the water over the work area.

8. Separate the snap-cap vials and tubes from the funnel.

9. Carefully remove the tube bundle and the attached polyacrylamide plug from the snap-cap vial.

10. Carefully separate the tube gels by pulling each tube away from the gel plug.

USING THE IEF TUBE GEL RUNNING SYSTEM
Preparing the Tube Gel Running System

1. Clean the running tank by rinsing with 18 MOhm water.

2. Prepare the anode and cathode solutions. (See page 25)

3. Carefully pour two liters of anode solution into the lower reservoir of the 1-D running tank. The temperature of the solution should be room temperature to prevent the precipitation of urea and OBG.

4. Soak the grommets and plugs in 18 MOhm water to remove any sodium hydroxide remaining from previous runs. The water will also act as a lubricant when inserting the tube gels into the grommets.

5. Place a grommet upside down on the bench top. Insert the top of a tube gel into the grommet and gently push the tube into the grommet.

6. Seat the preparative tubes with grommets in the inner circle of larger holes in the running rack. Fill any remaining preparative holes with preparative plugs.

7. Plug the smaller holes with analytical plugs.

8. Pour a small amount of the cathode solution into the upper reservoir to verify it is not leaking.

9. Once you determine there are no leaks, lower the 1-D running tank into the anode solution.

10. Fill the upper reservoir to a total of 800 ml of cathode solution.

11. To remove the bubbles in the top of the tube gels, fill a Hamilton syringe with cathode solution and attach the fine needle supplied with the system. Insert the needle to just above the surface of the gel, and fill the space with solution. This will displace the air bubbles.

Pre-focusing the Preparative Gels

NOTE: Experiments in the R & D laboratory (unpublished) have shown that eliminating the pre-focusing step results in increased protein resolution. If you wish to skip this step, proceed directly to the Sample Loading section.

To pre-focus preparative gels, follow the instructions on **"Pre-focusing the Gels using the power supply"** in the Analytical Gel section (page 15). For preparative gels the following parameters are recommended:

Number of gels:	Maximum of 8
Max voltage:	1000 V
Duration:	5 h
Max current:	600 µA

Loading the Sample

Remove the cover and use a 500 µl Hamilton syringe to apply up to 300 µl of sample to the top of each gel tube. For preparative gel electrophoresis, up to 1 mg of protein can be added to each gel.

Focusing the Preparative Gels

To focus preparative gels, follow the instructions for **"Focusing the Gels"** (page 18). For preparative gels the following parameters are recommended:

Number of gels:	8
Max voltage:	1500 V
Duration:	17 h, 15 minutes
Max current:	600 µA
Volt hours:	17,500 VH

PREPARING FOR 2-D ELECTROPHORESIS

Extruding and Equilibrating Preparative Gels

1. Remove the preparative gels after focusing and place them on ice for a minimum of five minutes to cool and stiffen. This makes the gels easier to extrude. It is normal for the urea and OBG to precipitate and turn white.

2. Load the preparative gel extrusion syringe with 18 MOhm water. Put the luer end of the tubing fitting on the luer tip of the syringe. Push the glass tube into the other end of the plastic tubing.

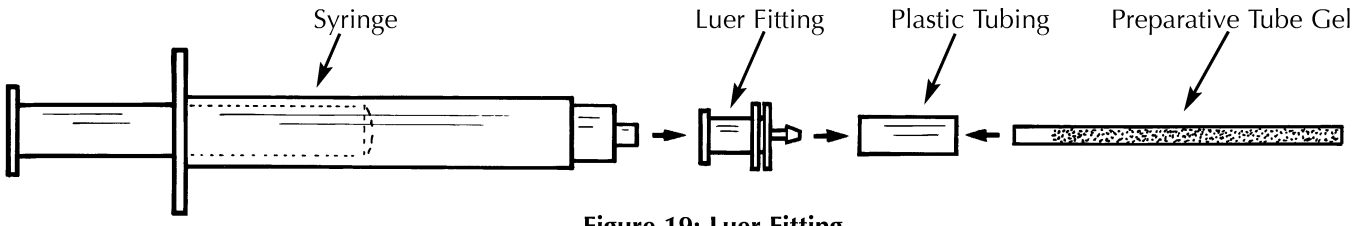

Figure 19: Luer Fitting

NOTE: If the preparative gels are to be frozen, freeze in a small amount of 10% glycerol. Store at -70° C.

3. Slowly apply pressure to the syringe and expel the gel from the tube into an equilibration tray with 10 ml of Equilibration Buffer I.

4. Incubate for 10 minutes on a shaker tray.

5. If you are planning to analyze proteins using mass spectrometry, pour off Equilibration Buffer 1 and repeat using 10 ml of Equilibration Buffer II in order to alkylate reactive free thiols.

6. Gels are now ready to be loaded on the 2-D gel. Please see page 24 of the 2-D Casting and Running Manual.

BUFFERS AND CASTING SOLUTIONS

Running Buffers

Cathode (Upper) Solution

Reagent	Final Concentration
10.0 ml 10 N NaOH (80-0075)	100 mM
Add the NaOH to 990 ml of 18 MOhm water	

Anode (Lower) Solution

Reagent	Final Concentration
1.4 ml 85% phosphoric acid (80-0074)	10 mM
Add the phosphoric acid into 2 L of 18 MOhm water.	

Solubilization and Rehydration Buffers

0070-3981 Urea Solubilization/Rehydration Buffer Components

Reagent	Amount	Final Concentration
Urea	48 g	8 M
CHAPS	2 g	2%
DTT	0.15 g	10 mM
Ampholytes (3-10)	2 ml	2%
Bromphenol Blue	0.010 g	0.01%
18 MOhm Water	to 100 ml	

Procedure

1. Add 50 ml of 18 MOhm water to a 100 ml graduated cylinder.
2. Place the cylinder on a magnetic stirrer.
3. Slowly add the urea to the water while stirring.
4. When all the urea has dissolved, add the other ingredients and stir until everything has dissolved.
5. Remove the magnetic stirring bar and adjust the volume to exactly 100 ml with 18 MOhm water.
6. Filter the solution through a 0.45 micron filter.
7. Store frozen at -70°C in 1 ml aliquots.

If there is a problem dissolving the urea, you may slightly warm the solution to 30° C. Exceeding this temperature may result in carbamylation of proteins.
This solution is used to solubilize the protein samples.

0070-4019 Urea/Thiourea Solubilization/Rehydration Buffer
Components

Reagent	Amount	Final Concentration
Urea	42 g	7 M
Thiourea	15.2 g	2 M
CHAPS	2 g	2%
DTT	1.0 g	65 mM
Zwittergent (3-10)	1.0 g	1%
Bromphenol Blue	10 mg	0.01%
Ampholytes (3-10)	0.8 ml	0.80%
18 MOhm Water	to 100 ml	

Procedure

1. Add 50 ml of 18 MOhm water to a 100 ml graduated cylinder.
2. Place the cylinder on a magnetic stirrer.
3. Slowly add the urea to the water while stirring.
4. When all the urea has dissolved, add the thiourea and stir well until dissolved.
5. Add the other ingredients and stir until everything has dissolved.
6. Remove the magnetic stirring bar and adjust the volume to exactly 100 ml with 18 MOhm water.
7. Filter the solution through a 0.45 micron filter.
8. Store frozen at -70°C in 1 ml aliquots.

If there is a problem dissolving the urea, you may slightly warm the solution to 30° C. Exceeding this temperature may result in carbamylation of proteins.

Aliquot and freeze. Thaw tubes at no more than 30° C to avoid formation of breakdown products of urea that could lead to carbamylation of proteins. This solution is used to solubilize the protein samples.

This product is supplied frozen and should remain frozen until used. The concentration of Zwittergent® 3-10 in the buffer is near its maximum solubility in the presence of high concentrations of urea; therefore, the buffer must be warmed to 30° C in order to re-dissolve all of its constituents. If all of the constituents are not dissolved, the solubilization of proteins in the samples will be less than optimal.

0080-0171 Sample Buffer III
Components

Reagent	Amount	Final Concentration
Urea	59.7 g	5 M
Triton X-100	4 ml	4%
Ampholyte Solution 3-10	5.5 mL	5.5%
DTT	1.54 g	10 mM
18 MOhm Water	to 100 ml	

Procedure

1. Add 50 ml of 18 MOhm water to a 100 ml graduated cylinder.
2. Place the cylinder on a magnetic stirrer.
3. Slowly add the urea to the water while stirring.
4. When all the urea has dissolved, add the other ingredients and stir until everything has dissolved.
5. Remove the magnetic stirring bar and adjust the volume to exactly 100 ml with 18 MOhm water.
6. Store frozen at -70˚C in 1 ml aliquots.

This solution is used to solubilize the protein samples and act as a vehicle for their transfer to the tube gels.

If there is a problem dissolving the urea, you may slightly warm the solution to 30˚C. Exceeding this temperature will result in carbamylation of proteins.

Equilibration Buffers

0080-0233 Equilibration Buffer I
Components

Reagent	Amount	Final Concentration
Urea	360 g	6 M
DTT	20 g	130 mM
Glycerol	300 ml	30%
Tris base	5.44 g	45 mM
SDS	16 g	1.6%
Bromphenol Blue	0.02 g	0.002%
Acetic acid, glacial	~15 ml	to pH 7
18 MOhm Water	to 1 L	

Procedure

1. Add 250 ml of 18 MOhm water and 300 ml of glycerol to a 1 L graduated cylinder.
2. Place the cylinder on a magnetic stirrer and stir until glycerol has dissolved.
3. Slowly add the urea to the water/glycerol solution while stirring.
4. When all the urea has dissolved, add the other ingredients except glacial acetic acid and stir until everything has dissolved.
5. Using a pH meter, adjust the pH of the solution to 7.0 with the glacial acetic acid. It will take approximately 15 ml.
6. Remove the magnetic stirring bar and adjust the volume to exactly 1 L with 18 MOhm water.
7. Store frozen at -70°C in 50 ml aliquots.

This buffer is used for equilibration of 1-D gels prior to loading onto the 2-D gel. It coats the separated proteins with SDS and reduces sulfhydral groups.

80-0232 Equilibration Buffer II
Components

Reagent	Amount	Final Concentration
Urea	360 g	6 M
Iodoacetamide	25 g	135 mM
Glycerol	300 ml	30%
Tris base	5.44 g	45 mM
SDS	16 g	1.6%
Bromphenol Blue	0.02 g	0.002%
Acetic acid, glacial	~15 ml	to pH 7
18 MOhm Water	to 1 L	

Procedure

1. Add 250 ml of 18 MOhm and 300 ml of glycerol to a 1 L graduated cylinder.
2. Place the cylinder on a magnetic stirrer and stir until glycerol has dissolved.
3. Slowly add the urea to the water/glycerol solution while stirring.
4. When all the urea has dissolved, add the other ingredients except glacial acetic acid and stir until everything has dissolved.
5. Using a pH meter, adjust the pH of the solution to 7.0 with the glacial acetic acid. It will take approximately 15 ml.

6. Remove the magnetic stirring bar and adjust the volume to exactly 1 L with 18 MOhm water.

7. Store frozen at -70° C in 50 ml aliquots.

This buffer is used for equilibration of IEF gels prior to loading onto the 2-D gel. It coats the separated proteins with SDS and alkylates the reduced sulfhydral groups. This is especially important if one will use mass spectrometry for protein characterization.

Gel Casting and Running Reagents

For extra convenience, the 1-D Analytical Casting Solution is also available in frozen aliquots.

0080-0091 1-D Analytical Casting Solution
Components

Reagent	Amount	Final Concentration
Urea	57 g	9.5 M
Triton X-100	2.0 ml	2.0% (v/v)
Duracryl (40% 19:1)	10.00 ml	4%
CHAPS	0.3 g	0.3%
18 MOhm water	to 95 ml	

Procedure

1. Add 40 ml of water, Triton X-100 and Duracryl to a 100 ml graduated cylinder and stir until thoroughly mixed.

2. Slowly add the urea and stir until everything is dissolved.

3. Add CHAPS and stir until dissolved.

4. Bring the volume up to 95 ml.

5. Pipette 5.65 ml aliquots into the snap-cap vials.

6. Store frozen at -70°C.

For extra convenience, the Preparative Gel Casting Solution is also available in frozen aliquots.

0080-0149 Preparative Gel Casting Solution
Components

Reagent	Amount	Final Concentration
Urea	120 g	8 M
Octyl-B-D-Glucopyranoside (OBG)	5 g	2.0%
3-10 Ampholytes	34 ml	5.5%
Duracryl™ (30T, 2.6C)	36.5 ml	4.4%
18 MOhm water	to 250 ml	

Procedure

1. Add 100 ml of 18 MOhm water to a 250 ml graduated cylinder. Add the ampholytes and Duracryl, and mix.
2. Slowly add the urea. Stir until dissolved.
3. Add OBG and stir.
4. Bring the total volume to exactly 250 ml.
5. Aliquot the final solution into 25 ml volumes in preparative 60 ml snap-cap vials.
6. Use the solution immediately or store frozen at -70°C.

IEF SUPPLIES

Save time by using Genomic Solutions rehydration, equilibration, casting and running buffers.

Solubilization and Rehydration Buffers for tube gels or pHlash Strips

	Part No.
Urea Solubilization/Rehydration Buffer, 10 X 1 ml	0070-3981

This buffer was previously called Urea Rehydration Buffer. This solution is used to solubilize protein samples, rehydrate pHlash strips and act as a vehicle for the transfer of the proteins into the pHlash strips or onto tube gels.

Urea/Thiourea Solubilization/Rehydration Buffer,10 X 1 ml 0070-4019

This buffer was previously called "Thiourea Rehydration Buffer." This solution is used to solubilize protein samples, rehydrate pHlash strips and act as a vehicle for the transfer of the proteins into the pHlash strips or onto the tube gels. The thiourea in this buffer has been shown to improve solubilization with certain proteins.

Sample Buffer III, 25 ml 0080-0171

Sample Buffer III is used to solubilize the protein sample and act as a vehicle for the transfer of the protein onto the tube gel.

Equilibration Buffers for tube gels or pHlash Strips

Equilibration Buffer I, 1 X 50 ml 0080-0233

This was previously called "IPG Equilibration Buffer I" and was packaged in 10 X 10 ml vials. It can be used to equilibrate either tube gels or pHlash strips before loading onto the second dimension using tricine or Laemmli chemistry. It coats the separated proteins with SDS and reduces sulfhydral groups.

Equilibration Buffer II, 1 X 50 ml 0080-0232

This was previously called "IPG Equilibration Buffer II" and was packaged in 10 ml vials. It can be used to equilibrate either tube gels or pHlash strips before loading onto the second dimension using tricine or Laemmli chemistry. It coats the separated proteins with SDS and alkylates the reduced sulfhydral groups. This is especially important if one will use mass spectrometry for protein characterization.

Chemicals	**Part No.**
Acrylamide stock solution 1L (30 T 2.6 C)	0080-0084
Ampholytes, pH 3-10, 40% solution: 25 ml	0080-0159
Ampholytes, pH 3-10, 40% solution: 5 ml	0080-0087
Ampholytes, pH 4-8, 40% solution: 25 ml	0080-0158
Ampholytes, pH 4-8, 40% solution: 5 ml	0080-0086
Urea, 100 grams	0080-0180
Urea, 1000 grams	0080-0070
Ammonium persulfate, 25 g	0080-0090
Bromphenol blue, 5 g	0080-0082
Triton X-100, 100 ml	0080-0072
Triton X-100, 1000 ml	0080-0178
Octyl B-D Glucopyranoside, 10 grams	0080-0176
Octyl B-D Glucopyranoside, 50 grams	0080-0177
Threaded tube, 26 cm, analytical (18 per pack)	0080-0032
Phosphoric acid (anode) 85% solution, 500 ml	0080-0074
Sodium hydroxide (cathode), 10 N, 500 ml	0080-0075
DTT, Dithiothreitol powder, 5 grams	0080-0089
DTT, Dithiothreitol powder, 25 grams	0080-0181
1-D Analytical Frozen casting solution (10 per pack)	0080-0091
1-D Preparative Frozen casting solution (2 per pack)	0080-0149
CHAPS detergent, 10 g	0080-0073
Components of 1-D Casting System	0080-0006
Casting funnel	0080-0029
Casting cylinder	0080-0030
Snap-cap vials (20 per pack)	0080-0031
Threaded analytical tubes, 26 cm (18 per pack)	0080-0032
Accessories for Preparative Tube Gels	
Preparative funnel	0080-0105
Snap-cap vials for preparative gels (20 per pack)	0080-0106
Preparative gel alignment guide (5 per pack)	0080-0108
2 mm preparative glass tubes (10 per pack)	0070-3698
3 mm preparative glass tubes (10 per pack)	0080-0109
Preparative Gel Extrusion Adapter	0080-0225
Pre-cast Tube Gels	
pH 3-10 Carrier Ampholyte IEF Tube gels, 1.0 mm x 18 cm (6 per pack)	0080-0118
pH 4-8 Carrier Ampholyte IEF Tube gels, 1.0 mm x 18 cm (6 per pack)	0070-3572
pH 3-10 Carrier Ampholyte IEF Tube gels, 2.0 mm x 18 cm (8 per pack)	0070-3926
pH 3-10 Carrier Ampholyte IEF Tube gels, 3.0 mm x 18 cm (8 per pack)	0070-3264

0080-0007 Components of IEF Tube Gel Running System

	Part No.
1-D Cover w/cable	0080-0033
1-D Running cylinder	0080-0034
1-D Running rack	0080-0035
Analytical plugs (15 per pack)	0080-0036
Analytical grommets (15 per pack)	0080-0037
Preparative plugs (8 per pack)	0080-0038
Preparative grommets (8 per pack)	0080-0039
Syringe for gel extrusion (2 per pack)	0080-0040
Adapter for gel extrusion	0080-0041
Grommet installer	0080-0042
Needle, Hamilton (2 per pack)	0080-0044
Equilibration trays (5 per pack)	0080-0045
Syringe w/needle (2″)	0080-0046