DIN PPP-T001-10

HPE-FlatTop Tower

User Manual

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HPE FlatTop Tower

The HPE-FlatTop Tower is used for one-dimensional and two-dimensional electrophoresis gels where multiple consistent runs are required. The unit consists of four horizontal electrophoresis chambers which are built as drawers into a metal housing. The instrument is run with an external power supply (gelcompany cat# PPP-001-TI) and thermostatic circulator (chiller) (gelcompany cat# PPC-100-TI).

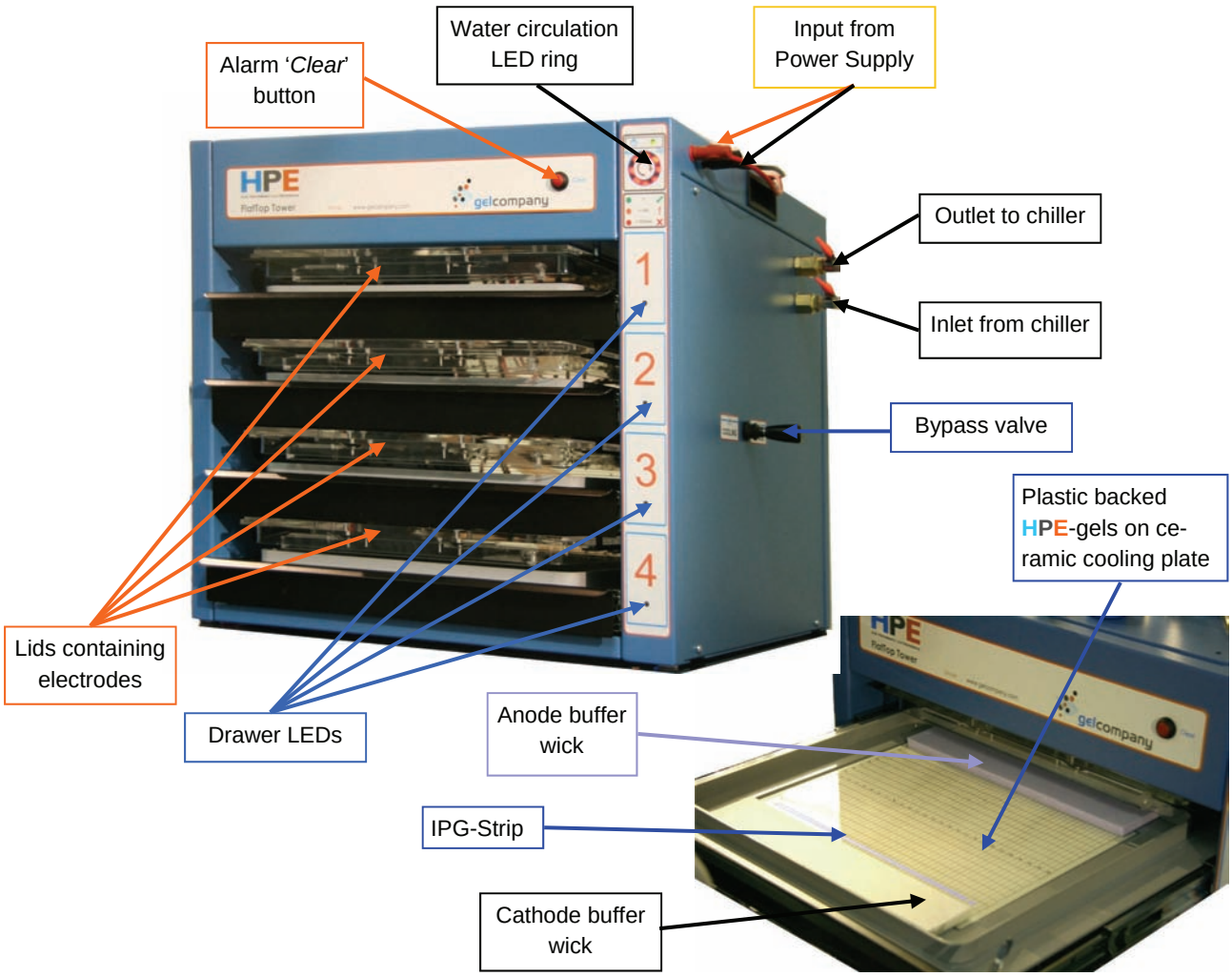
The gels are protected from light during the run, which prevents bleaching of fluorescent labels. No cassettes are required as the plastic-backed gels are run directly on an aluminium oxide ceramic cooling plates. The specially formulated HPE-gels allow high voltages to be used in the electrophoresis and when combined with the very thin gels (0.65mm) results in highly focused electrophoretic spots and bands. The patented cooling plates and inbuilt pump ensures efficient heat dissipation thus rapid and straight electrophoretic migration. An electronic sensor system delivers information about the electric field distribution between the gels, and indicates which drawer-chambers are in use. The system does not use buffer chambers; instead wicks are soaked with concentrated electrophoresis buffers and placed between the gel edges and the electrodes that are mounted into the lids. The electrode positions are adjustable to accomodate two different gel sizes. HPE-gels are available in two different sizes:

Standard size: 25 x 12.5 cm for:

- 1D SDS or native electrophoresis with 25, 52, and 103 sample wells
- 2D electrophoresis accomodating 2 x 11 cm IPG-strips and 1 MW standard lane
- 2D electrophoresis accomodating 3 x 7 cm IPG-strips and 2 MW standard lanes

Large size: 25 x 19.5 cm for:

- 2D electrophoresis with one 24 cm IPG-strip and 1 MW standard lane.

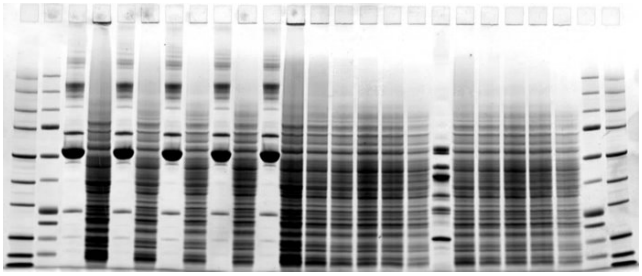


Examples of types of HPE-gels suitable for the FlatTop Tower

1D SDS PAGE 25 slots (cat# PCS-125-TK):

Marker, muscle, *E. coli* extracts

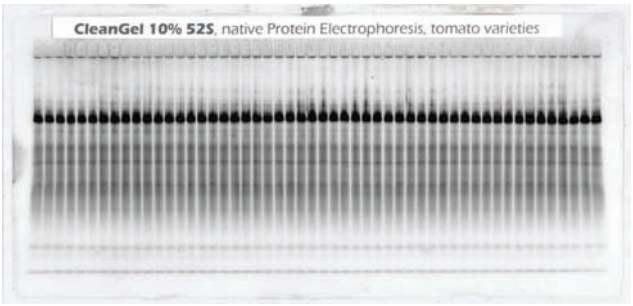
Coomassie™ staining



1D native PAGE 52 slots (cat# PCS-252-TK):

Tomato seeds

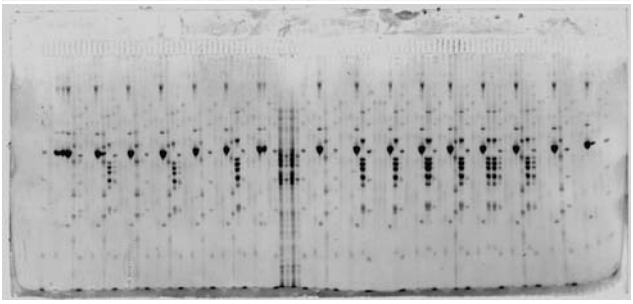
Coomassie staining



1D SDSPAGE 103 slots (cat# 1005-07):

Marker, serum, muscle, seed proteins

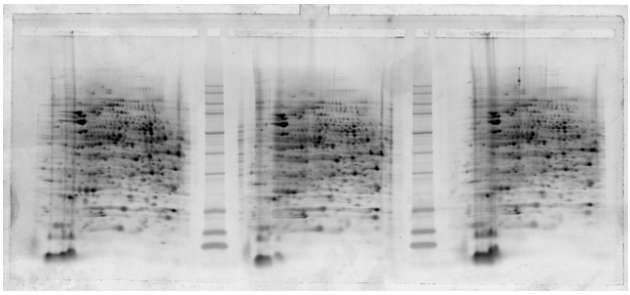
Coomassie staining



2D SDSPAGE running 3 IPG strips 7 cm "*Triple*" gel (cat# PCT-301-TK)

E. coli extracts

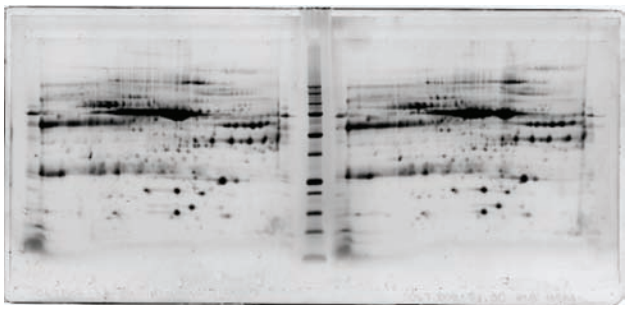
LavaPurple™ (cat# LP-011100) staining



2D SDSPAGE running 2 IPG strips 11 cm "*Double*" gels (cat# PCF-902-TK)

Human serum proteins

LavaPurple (cat# LP-011100) staining

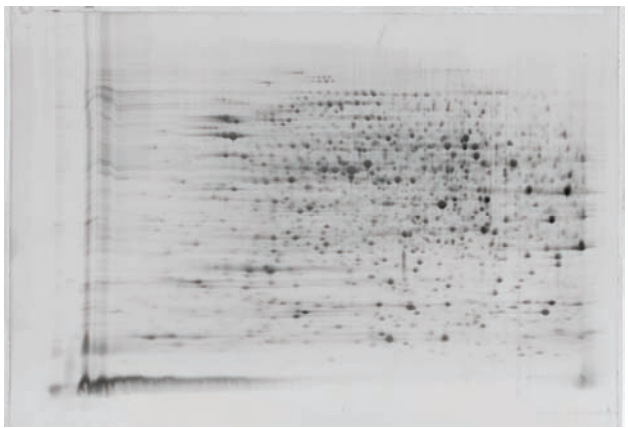


2D SDSPAGE 1 IPG strips 24 cm "*Large*" gels.

(cat# PCF-906-TK)

E.coli proteins

LavaPurple (cat# LP-011100) staining



Important Safety Information

PRIOR TO OPERATING THIS INSTRUMENT READ AND UNDERSTAND THIS MANUAL ENTIRELY.
THIS INSTRUMENT MUST BE USED ONLY BY INDIVIDUALS SKILLED IN ELECTROPHORESIS
THIS INSTRUMENT AND ITS COMPONENTS ARE FOR *IN-VITRO* RESEARCH USE ONLY

WARNING: OPERATION OF THIS INSTRUMENT REQUIRES HIGH VOLTAGE

- Disconnect the high voltage external power supply before opening any drawers.
- Turn off and disconnect any high voltage power supply before opening the safety enclosure lid.
- Disconnect the high voltage external power supply and AC mains supply before opening top panel.
- Disconnect the high voltage external power supply and the AC mains supply before cleaning or servicing.
- Do not spill or store liquids on the top of the unit.
- If liquid is observed to have spilled or overflowed into the electronics in the top of the **HPE**-FlatTop Tower, immediately disconnect the high voltage power supply and the AC mains power to the entire instrument before opening the safety enclosure lid.
- Do not operate or connect power sources to the equipment if there is any mechanical damage.
- Do not obstruct access to the controls (power switch and bypass valve), they must remain freely accessible while operating.

WARNING: THIS EQUIPMENT WEIGHTS 70KG

- The instrument requires appropriate lifting tools to locate into position.
- Do not attempt to lift without appropriate occupational health and safety considerations.
- Do not open multiple draws at once. The equipment may over-balance and either equipment damage or hazardous conditions may result.

WARNING: HIGH TEMPERATURE AND FIRE HAZARD

- High temperatures can be generated at the gel surface. Care should be taken when opening the drawer to asses the temperature before touching.
- Care should be taken not to allow the gel to dry out.
- Flammable gels must not be used.
- The equipment must be monitored by the operator at all times.
- Only use gels recommended for the **HPE**-FlatTop Tower.

This warning symbol highlights instructions that must be followed to avoid personal injury. It is important not to continue until all stated conditions are met and clearly understood.

ELECTRICAL REQUIREMENTS

Mains:	110 -120/220-240 V AC, 150VA max
DC (input):	0-1500V DC, 200mA max with 2mm to 2mm DC supplied cables only.
DC (input):	1-1000V DC, 200mA max with 2mm to 4mm DC cable only (accessory)

The supplied DC cables (2mm to 2mm connector type) are rated for 1500V. If cables or adaptors not supplied with the Tower are used ensure these have a suitable DC insulation compliance for the voltages used.

OPERATING CONDITIONS

Temperature: 5ºC to 40ºC. Altitude up to 2000m. Relative Humidity up to 80%.
It is not recommended to operate the system under the influence of extreme electromagnetic interference such as industrial mains noise, or extreme electrostatic discharges. Operating under these conditions may cause the front panel indicator or the +/- 20% current level indicator to show incorrect readings. Should this occur it is recommended to isolate or prevent the external interference effect and if necessary cycle the power switch on and off to reset the processor. It is recommended that the red and black leads from the power supply are twisted together to reduce possible RF-emissions and to avoid tangling.

In all cases of interference the unit remains CE mark compliant.

CE CERTIFICATION

This product meets the requirements of applicable CE-directives IEC 61010. A copy of the corresponding Declaration of Conformity is available on request. This CE approval is for the HPE FlatTop Tower only. The operator is responsible for maintaining the regulatory approval for the additional devices attached to the HPE-FlatTop Tower and CE mark approval for the system as a whole. This includes the DC power supply, DC cables and water cooler. In order to maintain CE mark regulatory requirements, if an alternate DC connecting cable is supplied by the user, it must remain less than 3 metres in length. Any alternate cables or adaptors used must have suitable insulation compliance. The supplied DC cable (2mm to 2mm connector type) is rated for 1500V. The optional 2mm to 4mm cable type can only be used with DC voltages up to 1000V.

The CE-symbol and corresponding declaration of conformity is valid for the instrument when it is:

- Used in indoors, in a laboratory location. Used with accessories described in this manual, or recommended by gelcompany.
- Used in the same state as it was delivered from gelcompany, except for alterations described in this manual.

Packing List

HPE-FlatTop Tower comprising:

- The Tower base unit
- 4 X lids packed separately containing the electrodes
- 1 X lamp for illumination of gels
- 1 mains cable
- 1 X 2mm power supply cables
- 2 X 2m PVC tubing with fabric net
- 4 X hose clamps for above tubing
- Handle for the bypass valve
- This manual.

HPE-FlatTop Tower Accessory Pack comprising:

- 4 PaperPools
- 1 Scalpel handle, pack of scalpel blades
- 2 forceps pointed, 2 forcepts blunt,
- 4 Falcon™ tubes
- 1 pair scissors
- 1 small roller

Please check the contents of your FlatTop Tower package and contact your local Gelcompany representative immediately if any items are missing.
In addtion to the FlatTop Tower you will need a suitable Power Supply and Chiller which are available from GelCompany.

If you have any comments on this manual, please send them to us at: Gel Company Pty Limited, Unit 3, 43 – 51 College Street, Gladesville NSW 2111 Australia.

1. Assembly, Installation and Running

Unpacking and Assembling of the HPE-FlatTop Tower

The HPE-FlatTop Tower is transported in protective packaging: The base unit and the 4 electrode lids are packed separately and require assembly at the final destination place. A mechanical lifting device and at least 2 people should be used to lift the FlatTop Tower. A ShockWatch label is included on the outside of the packaging which should be intact on arrival. Please inspect this and report if the ShockWatch labels show damage or are missing to your local gelcompany representative.

The Base-Unit

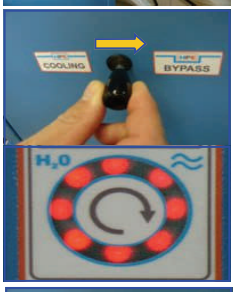
Place the base unit on the lab-bench but do not plug-in the power cable at this stage. With a spirit level, carefully level the base unit using the adjustable feet.
Remove the bypass handle and lamp from the accessories package .
Screw on the bypass valve handle.
Fix the lamp to the compartment lid using the two screws provided. Open the lid by removing the 2-screws and connect the lamp cable inside the compartment. Replace the lid screws.

Connecting the main power cable

Plug in the supplied power cable (220V, 110V) at the back of the base unit but do not switch on the FlatTop Tower at this point.

Connecting the Thermostatic circulator (Chiller)

It is important to place the chiller in a position that avoids warm air exhausting from the chiller heating the Flat-Top Tower. The tubing connecting the Tower to the chiller should be as short as possible and insulated with foam.
With the bypass valve set to "*bypass*," connect the FlatTop Tower to the chiller using the tubing provided. Fix the tubing using the hose clamps (Jubilee clips) provided. It is important these clamps are sufficiently tight to provide an air-tight seal. The chiller "*Outlet*" must be connected to the Tower "Inlet" and the chiller "*Inlet*" to the FlatTop Tower "Outlet". The flow-direction must be correct otherwise the cooling system will not function.



Removal of Air

For efficient cooling it is vital to remove any air from within the cooling plates in the Flat-Top Tower.
After connecting the FlatTop Tower, as described above, switch on the chiller with the valve in the '*Bypass'* position. Leave the chiller running until all of the air in the connecting pipes is removed, this may take 2-3 minutes.
Move the valve position from '*Bypass'* to '*Cooling'* and leave the chiller running until little or no air is seen in the connecting pipes. This will take around five minutes during which time you will need to refill the chiller reservoir as the FlatTop Tower takes up about 2 litres of water.
With cooling water through the system switch on the FlatTop Tower. The green light indicates that the power is on, the internal pump will be heard to start. The green H_2O LED ring will illuminate and rotate approximately 2 rotations per second. If the red, stationary LEDs are seen or the green LED rotates slowly something is wrong. Most likely this will indicate that the tubing from the chiller is connected back to front, is kinked, or the valve is in the '*Bypass'* position.
An alarm will sound if any drawers are open, this can be muted using the *'clear'* button and by pushing the drawer back into position.
To remove the last traces of air push the valve between "*Cooling"* and "*Bypass"*, creating a "*hammering"* effect that should remove any last traces or air. Watch the return tubing to the chiller to determine when the air has been fully removed. Leave running for 30 mins to ensure all traces of air have been removed.

Electrode lids

Take the electrode lids out of the package. Remove the protection film and paper.
Insert the lids into their "park" positions" below the drawers with the connecting plugs inside the FlatTop Tower.

Connecting the power supply

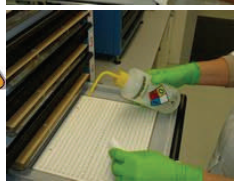
The power supply is connected with the FlatTop Tower using the black and the red cable supplied, do not mix these cables: connect the red cable to the red plug and the black cable to the black plug. When the cables are plugged in the wrong way, the electronic control in the FlatTop Tower will detect this and automatically disconnect the electric current.

For the HPE-Power Supply 1500 you will need to insert the adaptor provided into the end of the lead. The supplied DC cables (2mm to 2mm connector type) are rated for 1500V. If cables or adaptors not supplied with the Tower are used ensure these have a suitable DC insulation compliance for the voltages used.

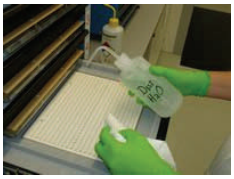
When running the Tower do not open a drawer until you have switched off (frozen/paused/standby) the power supply. Opening the drawers with the power supply running may damage your power supply.

Cleaning

To obtain the best results with the FlatTop Tower requires that it is scrupulously clean and we recommend cleaning before and immediately after use. When first running the FlatTop Tower, or if the cooling plates become soiled, they should be cleaned using a 0.1% SDS solution, followed by isopropyl alcohol and finally distilled water. Subsequent cleaning can involve cleaning with distilled water alone. The cooling plate can be gently rubbed and dried using a lint-free tissue. Abrasive cleaners and other solvents must not be used. The electrodes should be cleaned using a lint free tissue moistened with distilled water.

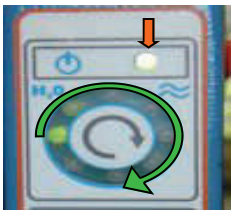


To minimize contamination (e.g. keratin) wear powder-free gloves, face masks, lab coat and a hair net. Handle wicks using bunt end and IPG-strips using pointed end forceps. For fluorescence labeling and staining use lint-free tissues and low fluorescence materials.



Switching on the Tower

The FlatTop Tower has a internal pump that must not be run dry. To avoid this always switch on the chiller first with the valve in the "*Bypass*" position. Allow the chiller to pump for 2-3 minutes with the valve in "*Bypass"* to ensure no air from the chiller is pumped into the cooling plates. When no air is apparent in the chiller tubing switch on Tower mains at the mains. The left green light indicates that the main power is on, the LED ring will show stationary red until the bypass valve is open. If there is an audible alarm ensure all drawers are closed and push the the red "Clear" button.



The bypass function

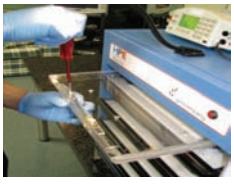
In horizontal electrophoresis gels are applied directly onto the cooling plate. If the gels are cooled before the lid is applied this can lead to condensation on their surface. Therefore before and during loading the gels, the bypass valve should be set to "*Bypass*". After gels, IPG-strips, electrode wicks, and electrode lids have been applied, the valve must be changed to "*Cooling*".

Switching off the FlatTop Tower

Switching off the FlatTop Tower should be done in the reverse sequence. Used wicks can be disposed of into normal waste. The cooling plate and electrodes should be immediately cleaned using a distilled water and a lint-free tissues. Do not leave gels, wicks or cooling fluid on the cooling plates as this can result in residues that are more difficult to remove.

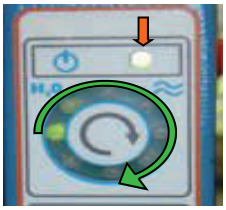
Care with the electrodes and changing electrode positions

The electrodes are carefully constructed from platinum wire and can be easily damaged if not handled correctly. To avoid damage store the electrode lids in the lid carriers contained in the drawer above. Never put the lids electrode side down on the bench as this may damage the platinum wire. Clean the platinum electrodes with a lint free tissue moistened with distilled water. Rub the tissue only up and down the electrode, never across the electrodes.



The electrode positions can be adjusted to two different gels sizes: '*standard'* and '*large'* size. When changing the positions, leave the electrode lid in the parking position to prevent damage to the electrodes. Be careful to catch the nut on the underside of the lid into which the screw fits.

Warning lights and alarms.



Green power light—mains power is connected and switched on.

Green rotating LEDs indicate water is flowing through the cooling plates.

Red stationary LEDs water is not flowing through the cooling plates.

Power supply alarms

The current flow in individual drawers in the Tower are monitored and different alarms indicate different problems. To facilitate monitoring there is a 33 second delay between switching on the power supply and illumination of the individual drawer lights.

Audible alarm alone that beeps every 4 seconds indicates that one or more drawers are open. The LED on open drawers will not be illuminated. Alarm can be muted by pushing the '*Clear'* button. Closing the drawer(s) should resolve the problem.

Green drawer LED indicates power is supplied to that drawer and that it is functioning correctly.

Audible alarm plus orange warning light on a specific drawer indicates that power to that drawer is 20% greater or less than the average for all four drawers. Power will continue to be supplied to the drawer and the alarm can be muted by pushing the '*clear'* button. Check the set-up of electrode wicks have been correctly positioned and correct buffers used. Check and ensure good connection between electrode and wicks. Check that the gels suitable for the FlatTop Tower and the same gel types have been used in all drawers. Check the correct (and same number) of electrode wicks applied to each gel. Ensure that the cooling plate is properly cleaned. Drawers can be run with the orange light illuminated but may show different migration rates.

Audible alarm plus red warning light indicates that the power to that the current to that drawer is >100mA. Power will be shut down to ALL drawers and will not reactivated until user intervention (drawer opened, power cycled.) This alarm will repeat if the issue has not been resolved. The power shut down is to avoid excessive heating of the gel that could be hazardous and or result in damage to the equipment. Users should identify and resolve problem before continuing with electrophoresis. Check the set-up, power supply and ensure that only gels recommended for use on the FlatTop Tower are used.

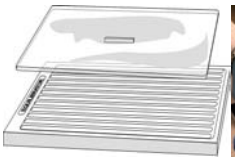
Running less than 4 gels

Between 1 and 4 gels can be run at any one time on the Tower. To avoid alarms sounding the electrode lids of drawers that are not in use should remain unplugged.

2.Loading and Running Gels

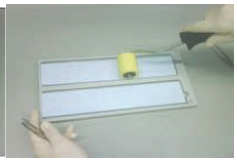
In this section general instructions for loading and running gels on the Flat-Top Tower are provided. Running conditions for specific types of gel are described in subsequent sections. A video and other useful information on running a the HPE-Tower is available for www.gelcompany.com.

Equilibrating IPG-strips




Gelcompany IPG strip equilibrator (cat# 1003-04) provides a convenient way to equilibrate IPG-strips. The equilibrator has been designed so that strips can be conveniently transferred from one slot the first equilibration solution (e.g. DTT) to the second (e.g. IAA).

Preparing Electrode Wicks

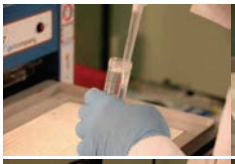
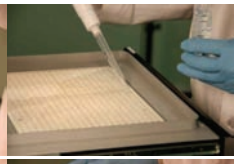



Electrode wicks, soaked in an appropriate buffer, provide a convenient alternative to buffer tanks. The wicks should be fully soaked for at least 15 minutes, thus we recommend you prepare the wicks first.

For large format gels multiple wicks (4) are used to provide sufficient buffer for a run. When using multiple wicks in a stack, air between the wicks can be removed by applying gentle pressure using a roller before the stacks are applied onto the gel. When using multiple wicks, soak the annodal and cathodal wicks in separate PaperPool rather than side-by-side in a single pool to avoid cross-contamination between the buffers.


The wicks should be moist but not dripping and sufficient buffer should be added so there is a small amount of buffer remaining in then gelcompany PaperPool (cat# 1003-03). Remove excess electrode buffer from the wicks by tilting the electrode wicks along one long edge and dab it on the paper pool bottom. When moving the wicks always hold them horizontal as holding them at a vertical angle can result in unequal buffer concentration.

Loading a gel

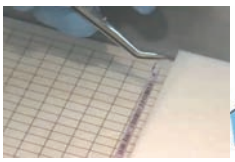





Place the valve is in the "*Bypass"* position to ensure that the gel is not cooled at this stage. To facilitate good contact a specially formulated cooling fluid is added to the surface of the cooling plate. This is then dispersed by sliding a gel from side to side using the gel bent into a "*U-shape*." The gel is then gently lowered avoiding air bubbles between the cooling plate and gel. The grid lines on the cooling plate are used to correctly locate the gel. Excess cooling fluid from around the gel is removed using a lint-free tissue.

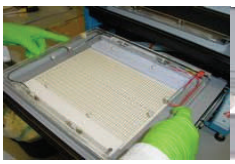
The electrode wicks are then applied with the cathode (white) at the front anode (blue) at the back. The electrode wicks should overlap the gel by at least 2mm. It is important that buffer is not dropped onto the gel surface and you should avoid moving the buffer soaked wicks over the gel. For the annodal wick take the wick around the back of the gel rather than over the top of the gel. The wicks should be rolled gently after positioning on the gel.

Applying the IPG-strip




The plastic film support on both sides of the IPG-strip must be trimmed just beyond the gel. The strip should be carried horizontally and applied to the slot centre first. The strip should be placed in the IPG slot, gel side down, with the anodal side to the right. To ensure good contact in the slot the back of the forceps are slid gently along the back of the IPG-strip .

Applying the lid




The lid containing the electrodes is slid from its parking position, carefully placed over the gel and plugged in at the back of the drawer. The valve set to '*cooling'* and the power supply switched-on. The power supply MUST be switched off or to standby (freeze/paused) before the drawers are opened.

3. One-Dimensional SDS Electrophoresis

1D SDS PAGE kits contains ready-to-use SDS polyacrylamide gels, running buffers, paper wicks, and sample diluters are available from **gel**company. The gels are polymerized on plastic backing, have a size of 25 x 12.5 cm X 0.45 mm thick and are available with either 25 X 15 µL slots (25S) X or 52 slots X 6 µL (52 S) slots. Various gel concentrations are available: 10% *T*; 12.5% *T*; 15% *T* . The gels are backed using a non-fluorescent film specifically designed for fluorescent pre-labelling of proteins (DIGE) and/or fluorescent staining (LavaPurple). This backing is also suitable for traditional staining methods such as Coomassie or Silver. For long shelf-life and optimal separation a Tris-Glycine gel chemistry is used which maintains the pH of the gel is below 7.

Sample pre-treatment: Double the sample volume by adding an equal volume of sample buffer (2X) then dilute the sample to acheive the an appropriate gel loading concentration (this dends on the sensitivity of staining method used e.g. Coomassie Blue, Silver Staining or LavaPurple) using 1 X sample diluter. Then reduce and alkylate your sample.

1. Switch the thermostatic circulator on, set to 15 °C. Switch the FlatTop Tower on and set the valve to "*By-pass*" to avoid water condensation on the gel surface.
2. Lay two electrode wicks into the compartments of the PaperPool. Apply 20 mL of the respective electrode buffer to each wick and allow to soak for at least 10 minutes (Fig 3.1).
3. Apply at least 3 mL cooling contact fluid onto the cooling plate.
4. Remove the gel from its packaging. Remove the cover-film. Grip the gel (surface-up) at the two lateral edges at the protruding film, bend it like an "*U*" and slide the film-backing left and right on the cooling plate to distribute the cool contact fluid evenly (Fig. 3.2). Remove excess cooling fluid along film edges with lint-free tissue paper.
5. Place the cathode strip onto the cathodal edge of the gel. The edge of the strip should overlap the gel not more than by 2 mm. Place the anode strip on the anodal edge and remove any air by gentle rolling.

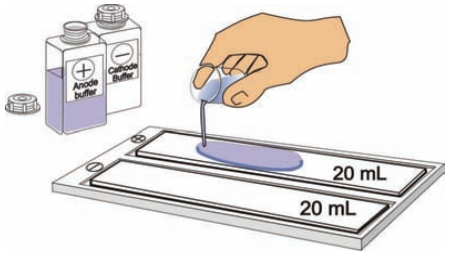


Fig. 3.1

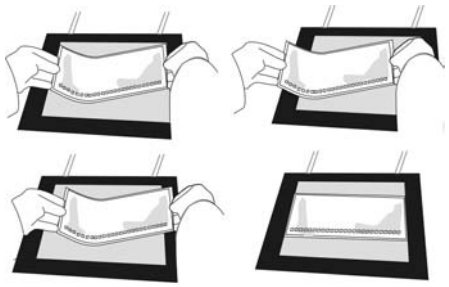


Fig. 3.2

6. Pipette 15 µL (25 S) or 6µL (52 S) of sample into the sample wells.
7. Clean platinum electrode wires before (and after) each electrophoresis run with moist tissue paper.
8. Close the lid while lowering the electrodes on the wicks, plug the cables in, turn the valve to cooling (15 °C)
9. Turn on your power supply and start the run according to table 3.1 or 3.2 depending on your sample type.

Table 3.1: Running conditions (15°C): Quick run for normal samples (total 2h)

Steps	Voltage	Current				Power				Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***1 gel***	***2 gels***	***3 gels***	***4 gels***	
S1	600 V ††	42 mA †	84 mA †	126 mA †	168 mA †	30 W ††	60 W ††	90 W ††	120 W ††	1 h
S2	1000 V ††	50 mA ††	100 mA ††	150 mA ††	200 mA ††	60 W †	120 W †	180 W †	240 †	1 h

Table 3.2 Running conditions (15°C): Slow run for difficult samples (total about 2 h 30 min)

S1	250 V ††	30mA †	60 mA †	90 mA †	120 mA †	10 W ††	20 W ††	30 W ††	40 W ††	45 min
S2	700 V ††	42 mA ††	84 mA ††	126 mA ††	168 mA ††	30 W †	60 W †	90 W †	120 W †	45 min
S3	1000 V ††	50 mA ††	100 mA ††	150 mA ††	200 mA ††	60 W †	120 W †	180 W †	240 W †	1 h

For programming BioRad Power Supplies only:

† - Set as "*constant*"

†† - Set as "*limit*"

4. Two-Dimensional Electrophoresis - "*Double*" and "*Triple*" Gels

Always wear powder free disposable gloves.

Important: Only use the gelcompany buffer kit for the running buffers and equilibration solutions.

1. Prepare the two equilibration solutions from the gelcompany IPG Strip equilibration buffer (Eq. buffer):
   DTT solution: Weigh urea and dithiothreitol (DTT) and add the equilibration buffer according to table 4.1 and dissolve completely.
   IAA solution: Weigh urea and iodoacetamide (IAA) and add the equilibration buffer according the table 4.1 and dissolve them completely.

Table 4.1. Preparing the equilibration solutions for 11 and 7 cm IPG strips:

Number X Size of strips	Urea [g]	DTT [mg]	IAA [mg]	Eq. Buffer [mL]	Total volume [mL]
2X11cm or 3X7cm	1.8	50	-	5	6
	1.8	-	125	5	6
4X11cm or 6X7cm	3.6	100	-	10	12
	3.6	-	250	10	12
6X11cm or 9X7cm	5.4	150	-	15	18
	5.4	-	375	15	18
8X11cm or 12X7cm	7.2	200	-	20	24
	7.2	-	500	20	24

2. Equilibrate each strip (gel-side up) in 3 mL (11 cm strips) or 2 mL (7 cm strips) solution in an equilibrator (fig. 4.1) on an orbital shaker with 30 rev/min:

Step 1	in DTT solution	for 15 min
Step 2	in IAA solution	for 15 min

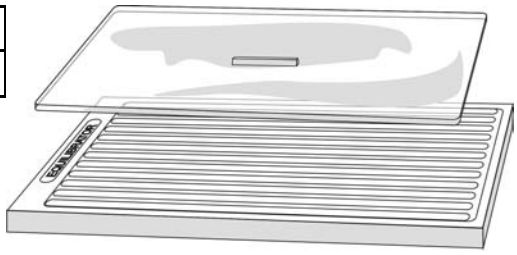


Fig. 4.1

3. After the 2nd equilibration discard the solution.

4. Apply 20 mL of each electrode buffer to the respective electrode wick in the compartments of the gelcompany PaperPool (fig. 4.2).

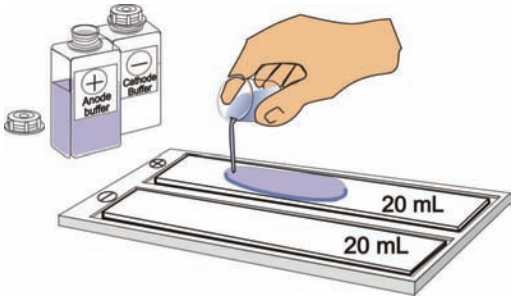


Fig. 4.2

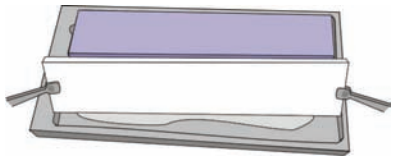


Fig. 4.3

5. Remove excess electrode buffer from the wicks by tilting the electrode wicks along one long edge and dab it on the PaperPool bottom (fig. 4.3).

6. Apply at least 3 mL of cooling contact fluid onto the cooling plate for good cooling contact.

7. Switch the thermostatic circulator on, set to 15°C. Switch the FlatTop Tower on and set the valve to "*By-pass*" to avoid water condensation on the gel surface.

9. Grip the gel (surface-up) at the two lateral edges at the protruding film, bend into a "*U-shape*" and slide the film-backing left and right on the cooling plate to distribute the cool contact fluid evenly (Fig. 4.4).

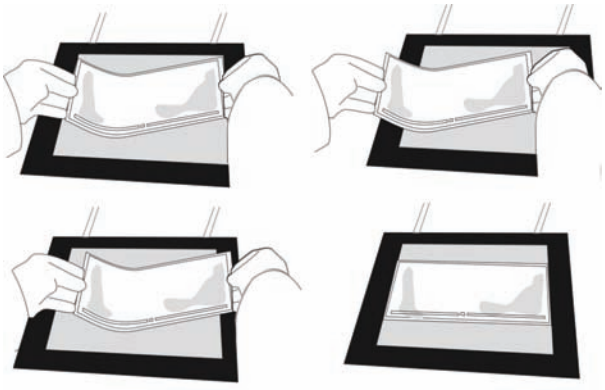


Fig. 4.4

10. Place the gel onto the cooling plate: the IPG strip-slot towards the cathode, the cathodal edge of the IPG strip-slot matching line "15".

11. Remove excess cooling fluid along the film edges with lint-free paper tissue.

12. Place the electrode wicks onto the gel edges overlapping them by at least 2 mm. Hold wicks horizontally, never at an angle as this causes unequal buffer concentration along the wick. Smooth out air bubbles with a roller.

13. Trim the film support of the IPG strips on both sides. Place the IPG strips gel-side down, anodal sides to the right, into the slots of the SDSGel and push them carefully towards the anode edges of the slots (fig. 4.5). Gently slide along the backing of the strips with the forceps to ensure good contact to the bottom of the slots.

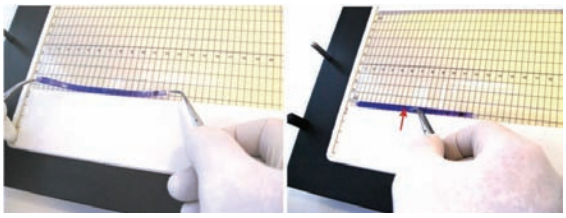


Fig. 4.5

14. Apply 5 µL SDS marker proteins to the marker well(s).

15. Close the lid while lowering the electrodes on the wicks, plug in the cables, switch the valve to "*cooling*" (15°C), switch on the FlatTop Tower main switch (pump and electronic control), and start the run according to table 4.2.

16. After 1 hour 10 min interrupt the run, remove the IPG strip(s), and then continue the run.

Running conditions: See table 4.2 for the maximum settings.

Table. 4.2: Running conditions (15 °C)

Steps	Voltage	Current				Power				Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***1 gel***	***2 gels***	***3 gels***	***4 gels***	
S1	100 V †	7mA ††	14mA ††	21mA ††	28 mA ††	1 W ††	2 W ††	3 W ††	4 W ††	30 min
S2	200 V †	13 mA ††	26mA ††	39 mA ††	52 mA ††	3 W ††	6 W ††	9 W ††	12 W ††	30 min
S3	300 V ††	20mA †	40 mA †	60 mA †	80 mA †	5W ††	10 W ††	15 W ††	20 W ††	10 min
after this step: **remove the IPG strips.**										
S4*	1000 V ††	40 mA †	80 mA †	120 mA †	160 mA †	25 W ††	50 W ††	75 W ††	100 W ††	2h*

* valid for homogeneous gels, for the gradient gel 10 -15% this step 4 takes **2.5 h** .

For programming BioRad Power Supplies only:

† - Set as "*constant*"

†† - Set as "*limit*"

NB: It is important not to use standard paper wicks for running gels if you are silver staining.

Please contact support@gelcompany.com for silver staining compatible electrode wicks.

5. Two-Dimensional Electrophoresis - Large Gels

Always wear powder free disposable gloves.

Important: Only use the gelcompany buffer kit for the running buffers and equilibration solutions.

1. Prepare the two equilibration solutions from the gelcompany IPG-Strip equilibration buffer (Eq. buffer):

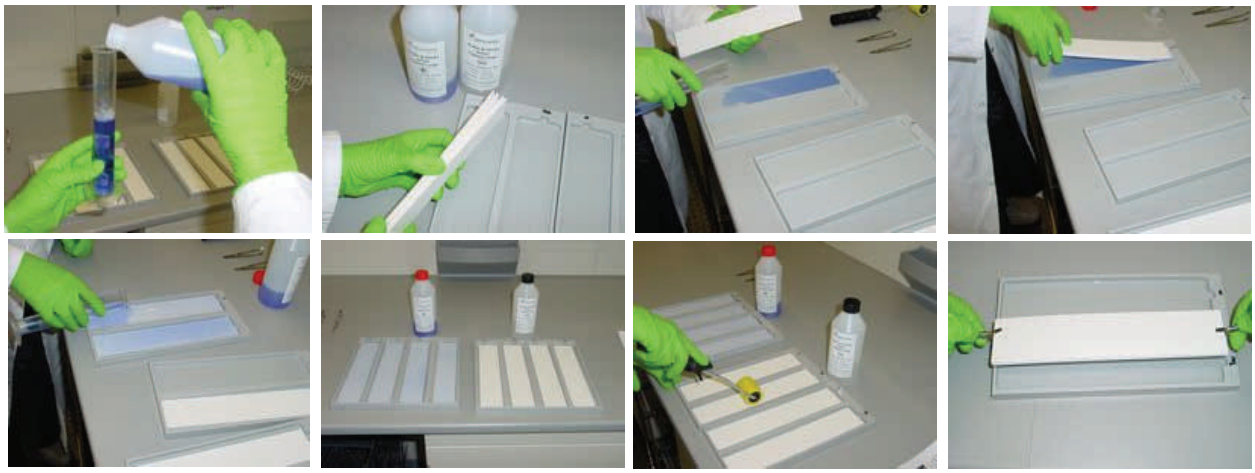


Fig. 5.1: Soaking stacks of four paper wicks in 80 mL electrode buffer. First pour 40 mL buffer into the empty PaperPool compartment, then lay the stack of paper wicks into the PaperPool and add another 40 mL buffer.

2. Equilibrate each IPG-strip (gel-side up) in 6 mL solution in a gelcompany equilibrator (cat# 1003-04; fig. 5.2) on an orbital shaker with 30 rev/min:

3. Apply 80 mL (2 x 40 mL) of each electrode buffer to the respective stack of four electrode wicks in the compartments of the gelcompany PaperPool (fig. 5.1) and soak for a minimum of 15 minutes.

***DTT solution**:* Weigh urea and dithiothreitol (DTT), and add the equilibration buffer according to table 5.1 and dissolve completely.
***IAA solution**:* Weigh urea and iodoacetamide (IAA), and add the equilibration buffer according the table 5.1 and dissolve them completely.

Table 5.1. Preparing the equilibration solutions for 24 cm IPG strips:

Number of strips	Urea [g]	DTT [mg]	IAA [mg]	Eq. Buffer [mL]	Total volume [mL]
1	1.8	50	-	5	6
	1.8	-	125	5	6
2	3.6	100	-	10	12
	3.6	-	250	10	12
3	5.4	150	-	15	18
	5.4	-	375	15	18
4	7.2	200	-	20	24
	7.2	-	500	20	24

After the 2nd equilibration step discard the solutions.

Step 1	in DTT solution	for 15 min
Step 2	in IAA solution	for 15 min

NB: It is important not to use standard paper wicks for running gels if you are silver staining.

Please contact support@gelcompany.com for silver staining compatible electrode wicks.

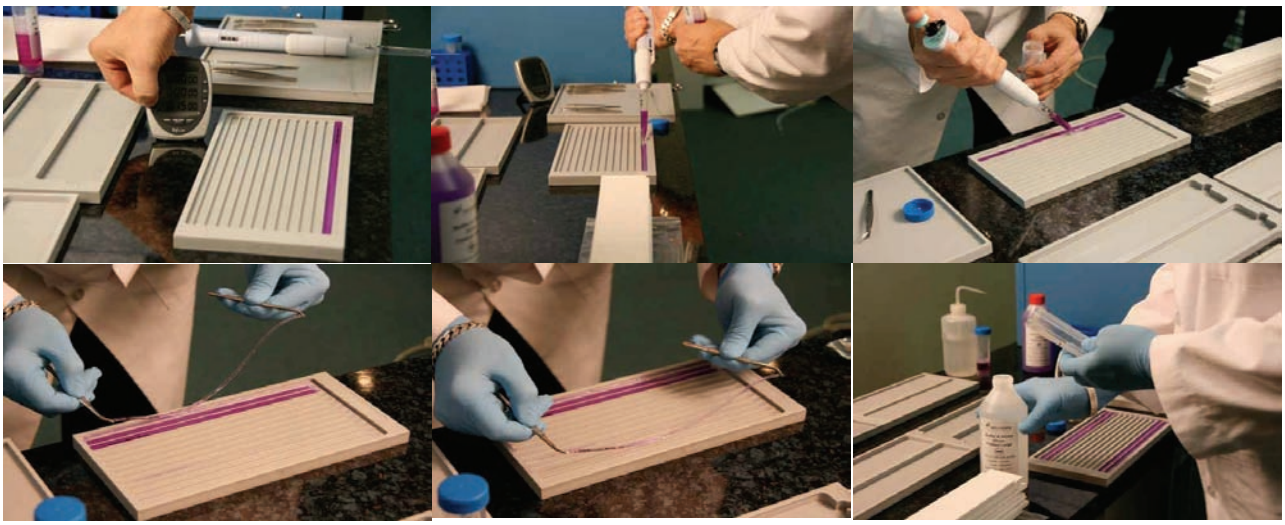


Fig. 5.2: Equilibration of the IPG-strips in 6 mL equilibration buffer.

3. Switch the thermostatic circulator on, set it to 5°C. Switch the FlatTop Tower on, and set the valve to "*Bypass*" to avoid water condensation on the gel surfaces.

4. Apply at least 4mL of cooling contact fluid onto the center of the cooling plate (fig. 5.3)

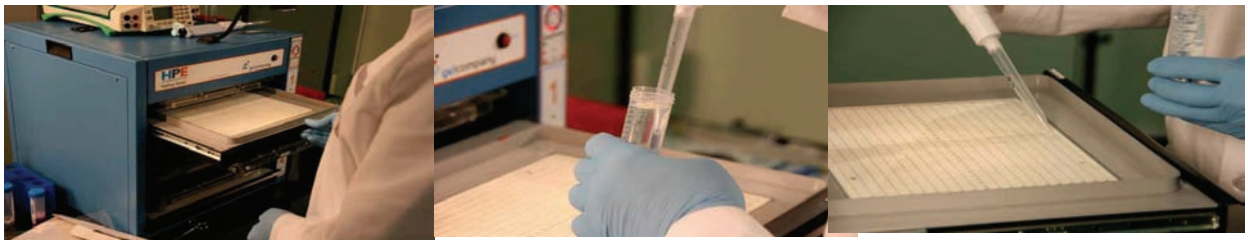


Fig. 5.3: Application of the cooling fluid

5. Grip the gel (surface up) at the two lateral edges at the protruding film, bend into a "*U-shape*" and slide the film-backing left and right on the cooling plate to distribute the cool contact fluid evenly (Fig. 5.4).

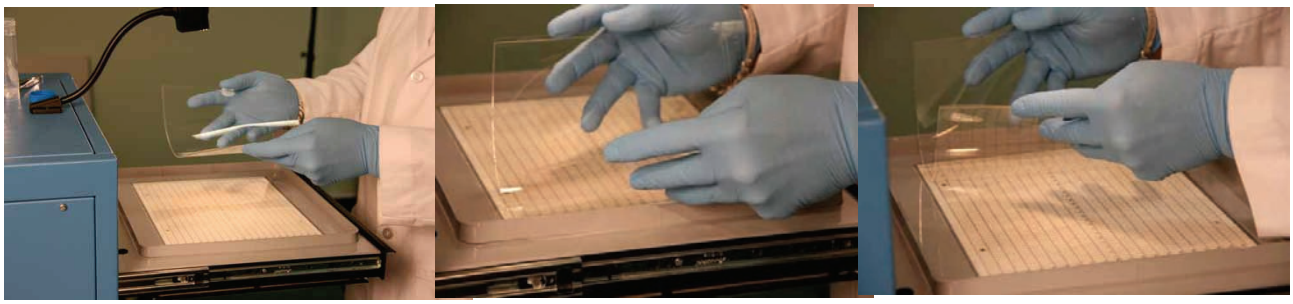


Fig. 5.4: Placing the gel on the cooling plate.

6. Remove excess cooling fluid along the film edges with lint-free paper tissue.

7. Finally lower the the gel onto the cooling plate: the IPG strip-slot towards the cathode, the cathodal edge of the IPG strip-slot matching line "*19*" (fig. 5.5).

Fig. 5.5: Placing Flatbed *Large* gel on the cooling plate.

8. Remove excess of electrode buffer from the wicks by tilting the electrode stack along one long edge and dab it on the PaperPool bottom (fig. 5.6). Always hold the wicks horizontally, do not tilt them, this would cause a higher buffer concentration on one side .

Fig. 5.6: Removal of excess buffer from the wicks. Always hold the wicks horizontally.

9. Place the stack of electrode wicks onto the gel edges, overlapping the gel by at least 2 mm. Smooth out air bubbles using a roller. Move the anodal wicks around the gel area to avoid losing buffer drops on the gel surface (fig. 5.7)

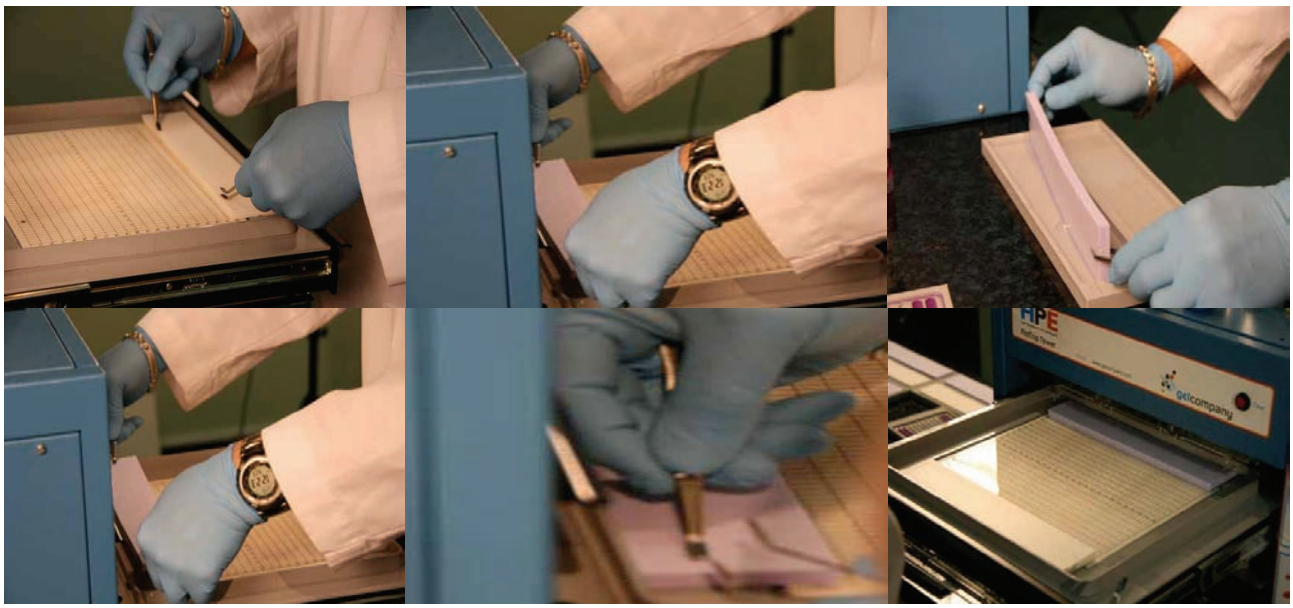


Fig. 5.7: Applying the stack of electrode wicks on gel edges.

10. Trim the film support of the IPG-strips on both sides.

11. Place the IPG strip gel-side down anodal side to the right, into the slot of the Flatbed Gel (fig. 5.8). Start in the middle to prevent accumulation of buffer towards one side. Push it carefully towards the anode edge of the slot. Slide along the backing of the strip with the forceps to ensure good contact to the bottom of the slot.

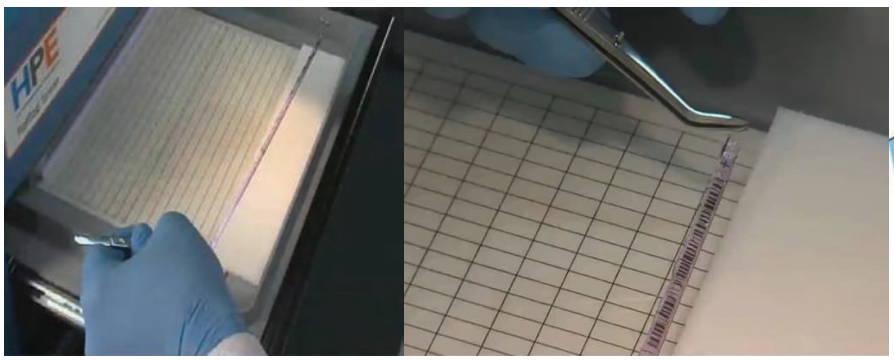


Fig. 5.8: Application of the IPG strip into the slot of the 2DGel flatbed. Always hold the strip horizontally.

12.Apply 5 µL SDS marker proteins to the marker well.

13. Place a glass plate on top of the electrode wicks (optional) stacks forming a bridge, this ensures an absolutely straight front. (fig. 5.9).

Fig. 5.9: Placing a glass plate on the buffer wicks for optimal contact of wicks and gel and to ensure a straight front.

14.Close the lid while lowering the electrodes on the wicks, turn the valve to "*cooling*" (15 °C), switch on the FlatTop Tower main switch (pumps and electronic control) and start the run according to tables 2 or 3 (*see below*.)

15. After 1 hour 10 min interrupt the run by standby/pausing/freezing the power supply, opening the drawers and remove the IPG-strip(s) and continue the run.

Tab. 5.2: Running conditions (day run), cooling temperature 15 °C

Steps	Voltage	Current				Power				Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***1 gel***	***2 gels***	***3 gels***	***4 gels***	
S1	100 V †	7mA ††	14mA ††	21mA ††	28 mA ††	1 W ††	2 W ††	3 W ††	4 W ††	30 min
S2	200 V †	13 mA ††	26 mA ††	39 mA ††	52 mA ††	3 W ††	6 W ††	9 W ††	12 W ††	30 min
S3	300 V ††	20 mA †	40 mA †	60 mA †	80 mA †	5 W ††	10 W ††	15 W ††	20 W ††	10 min
after this step: **remove the IPG strip.**										
S4	1500 V ††	40 mA †	80 mA †	120 mA †	160 mA †	30 W ††	60 W ††	90 W ††	120 W ††	3h 50min*

* valid for homogeneous gels, for the gradient gel 10-15 this step 4 takes **4 h 50 min**.

Tab. 5.3: Running conditions over night for homogeneous gels**, cooling temperature 15 °C

Steps	Voltage	Current				Power				Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***1 gel***	***2 gels***	***3 gels***	***4 gels***	
S1	100 V †	7mA ††	14mA ††	21mA ††	28 mA ††	1 W ††	2 W ††	3 W ††	4 W ††	30 min
S2	200 V †	13 mA ††	26 mA ††	39 mA ††	52 mA ††	3 W ††	6 W ††	9 W ††	12 W ††	30 min
S3	300 V ††	20 mA †	40 mA †	60 mA †	80 mA †	5 W ††	10 W ††	15 W ††	20 W ††	10 min
after this step: **remove the IPG strip.**										
S4	220 V ††	5 mA †	10 mA †	15 mA †	20 mA †	3 W ††	6 W ††	9 W ††	12 W ††	15 hours

**** Please note:** It is **not** recommended to run the gradient gel 2DGel flatbed 10-15 overnight.

For programming BioRad Power Supplies only:

† - Set as "*constant*"

†† - Set as "*limit*"

6. Staining of Flatbed Gel - Fluorescent

LavaPurple™ Staining

Below is a protocol for LavaPurple staining optimised for film-backed gels which longer steps than slab gels without backing because the solutions can enter the gel only from one side. A more detailed protocol along with other useful information can be obtained from the **gel**company website www.gelcompany.com.

LavaPurple staining must be performed in a clean plastic tray. We recommend against using trays that have been used previously for other stains (e.g. Sypro™ Ruby, Coomassie™) Transparent trays are OK. Do not use metal trays.

Solutions and Buffers

Solution 1 (fixation and acidification): Place 850mL of high purity water into a 1L bottle then add the contents of packet 'A', and mix until dissolved. Add 150mL of 100% ethanol and mix thoroughly.

Solution 2 (staining buffer): To prepare the staining buffer add 1L of high purity water to a 1L bottle and add the contents of packet 'B'. Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) then add 3.85g of packet 'C' and continue stirring until fully dissolved.

Solution 3 (washing): Mix 850mL of high purity water and 150mL of 100% ethanol in a 1L bottle.

Solutions 1-3 can be stored at room temperature and are stable for up to 6-months.

Table 6.1: **LavaPurple staining of 0.65 mm thick film-backed polyacrylamde gels**

Process	Solution	Volume per gel		Time
		Standard	Large	
1. Pre-fixing	Solution 1 (Dispose of this solution after use)	300 mL	400 mL	30 min
2. Fixing	Solution 1 Solution 1 from this step may be reused for acidification (step 5)	300 mL	400 mL	30 - 45 min
3. Staining	0.5 % (v/v) LavaPurple in Solution 2	300 mL 1.5 mL LavaPurple	400 mL 2.0 mL LavaPurple	1.0 - 2.0 hr
4. Washing	Solution 3 Solution 3 may be re-used up to 2 X	300 mL	400 mL	30 - 45 min
5. Acidify	Solution 1	300 mL	400 mL	30 - 45 min

Notes:

The gels must be completely covered with the various solutions. Staining (plastic) trays should be selected that are only marginally larger than the gels and gels should not stick to the bottom during staining.

Gels should be placed gel-side down in the staining solution.

Minimize carry-over of solutions between steps.

Orbital shakers (if available) provide the best results.

The gel may be left in solution 1 overnight with no negative effects.

Bring dye concentrate to room temperature and mix thoroughly prior to use.

Add LavaPurple concentrate to solution 2 before pouring onto the gel to prevent staining artifacts.

The staining solution must be made fresh (not more than 30 minutes prior to use).

The staining solution can be reused once for staining within 2 hours provided it is still purple in colour.

The fixing solution (step 2) can be re-used for acidification (step 5).

Do not stain longer than 3 hrs as signal will decrease after this time. If there is no time for scanning you can leave the gel in the acidifier (solution 1) overnight.

LavaPurple stained gels can easily be post-stained with semi-colloidal Coomassie Blue staining or silver staining for manual spot picking (see page 15).

Sypro is a trademark of Invitrogen

7. Scanning of Backed Gels - Fluorescent

The gelcompany Gel ScanFrame (cat# PAS-001-TI) is designed to prevent the curling of plastic backed gels during imaging. It is particularly useful for the scanning of fluorescently stained or labelled gels as this can take a considerable length of time during which the gels may dry and begin to curl.

The ScanFrame comprises a set of 3 x 2 non-fluorescent PVC-frame-bars to prevent curling of plastic backed gels during scanning (fig. 7.1).

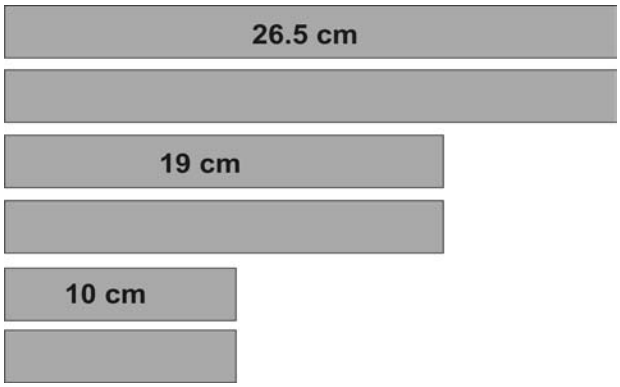


Fig. 7.1: The ScanFrame consist of
two 10 x 25 x 265mm;
two 10 x 25 x 190mm;
two 10 x 25 x 100mm
PVC-bars.

It is suitable of both gelcompany large (25 x 19.5 cm) and standard (25 x 12.5 cm) format, horizontal and vertical 2DGels, SDSGels, DNAGels and FocusGels

These bars are placed on the edges of the gel to press the whole gel horizontally on the scanner platen (fig. 7.2).

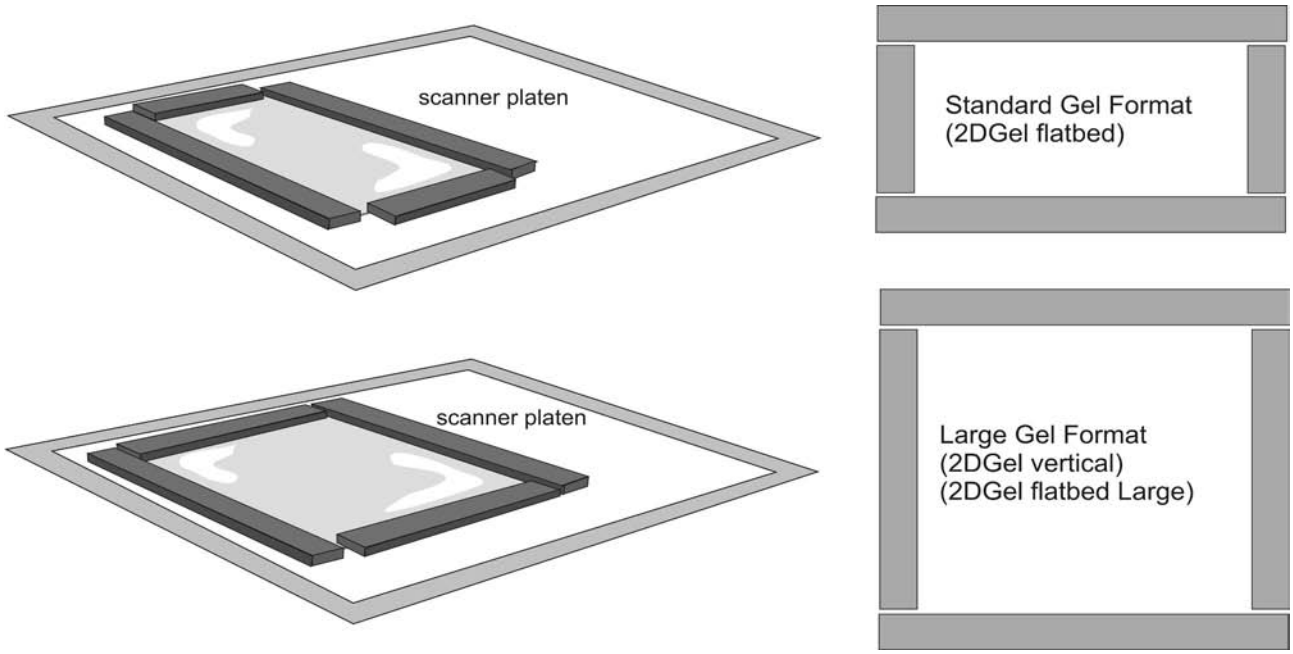


Fig. 7.2: The ScanFrame holding down a normal (25 x 12.5 cm) and a large sized gel (25 x 19.5 cm)

The gels are scanned with the gel surface down facing the platen directly after applying a few mL water on it (focal plane 0 or platen). During scanning the *ScanFrame* is laid on the gel to avoid curling of the edges. Do not apply "Press Sample."

For accurate spot picking the scanning orientation must be flipped as shown here:

Scanner Settings

Excitation maxima:

390 and 500nm, Common excitation sources include: UVA or 473 nm, 488 nm, and **532 nm** lasers.

Emission maxima: 610 nm. Common filters: 560 LP, 610 BP30, 600 BP10

Example: using Typhoon ™: 532 nm laser, 540 PMT, 610BP30 filter, 100 µm resolution, normal sensitivity.

8.Staining of Flatbed Gels - Colorimetric

8.1 Semi-colloidal Coomassie Staining

Semi-colloidal Coomassie staining is fixing and staining proteins at the same time. It is also very efficient for the detection of small peptides. It works best with Coomassie R 350, which is available in tablet form from GE Healthcare (17-0518-01). Also standard Coomassie Brilliant Blue R 250 can be used, but it will lead to a higher background. The procedure works best in a steel tray with grid. Three different types are available from gelcompany: standard, large, and the MultiStainer for staining up to 6 gels at a time.

Fig 8.1 Steel Tray with grid for standard size gels, gelcompany (cat.# 1003-25),

Fig 8.2 Steel Tray with grid for Large size gels, gelcompany (cat.# 1003-26). Suitable for *Large* and *Standard* gels

Fig 8.3 MultiStainer with grid for staining up to 6 gels (PGS-001-TI). Suitable for *Large* and *Standard* size gels

Stain with 0.01 % (w/v) Coomassie R 350 in 0.5% (w/v) Phosphoric acid / 3% (v/v) acetic acid using the MultiStainer:

Dissolve 1 tablet Coomassie R 350 in 2.8 L H_2O_{dist}, add 18 mL *o*-Phosphoric acid and 90 mL acetic acid, fill up to 3 L with distilled H_2O and add to the MultiStainer. Prepare a further 3 liters of Coomassie stain (as above) and add this to the Multistainer to bring the total to 6 L.

Staining:

1. Heat staining solution to 40°C in steel tray (Fig. 8.4) or MultiStainer for multiple gels (Fig. 8.5) with grid while stirring

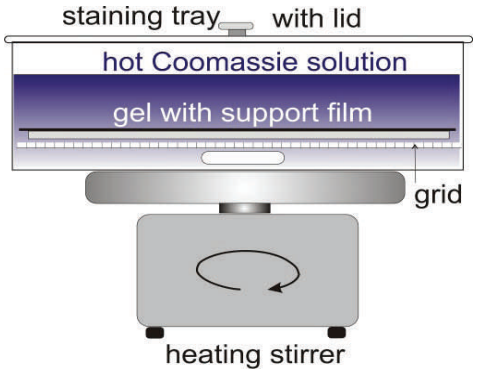


Fig. 8.4: Set-up for hot Coomassie staining.

Single gel in a steel tray with grid

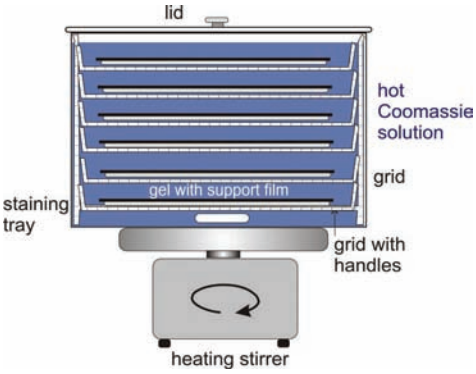


Fig. 8.5: Set-up for hot Coomassie staining.

Multiple gels in a Multistainer

2. Place gel with **gel-side down** on the grid and stain for 2 hours - overnight.

Destaining:

De-stain background for several hours in 10 % (v/v) acetic acid (a few changes) at room temperature in a plastic tray on a shaker.

Imaging.

Gelcompany ScanFrame (cat# PAS-001-TI) is also useful to prevent curling during the imaging of Coomassie stained gels

Note: This CBB staining method can be used as a fixing procedure prior to silver staining. After de-staining the background continue directly with the sensitizer step.

8.2 Mass Spectrometry compatible Silver Staining

A number of different Silver Staining methods are available. The method below combines has been selected due to its MS-compatibility and has been optimised for staining of backed gels. It is important not to use standard paper wicks for running gels if you are silver staining. Please contact support@gelcompany.com for silver staining compatible electrode wicks.

Note: Film-backed gels need more and longer steps than slab gels without backing, because the solutions can diffuse into the gel only from one side.

Table 8.2: MS compatible Silver staining of 0.65 mm thin film-backed polyacrylamide gels

step	reagent	volume	time
2 x Fixing	10% acetic acid, 40% ethanol, 1% citric acid *(25 mL acetic acid, 37.5 ml ethanol, 2.5 g citric acid)*	250 mL	2 x 30 min (or 2nd overnight)
4 x Washing	H_2O dist	4 x 250 mL	4 x 5 min
If the gel has been prestained with LavaPurple or semicolloidal Coomassie Staining, fixing and washing is not necessary, start directly with the sensitizing step:			
Sensitizing	0.025 % (w/v) Na-thiosulphate, 10 mM Na-acetate, 30 % ethanol *(63 mg Na-thiosulfate, 350 mg Na-acetate, 75 ml EtOH)*	250 mL	40 min
4 x Washing	H_2O dist	4 × 250 mL	4 × 5 min
Silvering*)	0.2 % $AgNO_3$ /0.03% formaldehyde (w/v) *(500 mg $AgNO_3$ / 225 µL formaldehyde (37%) *)*	250 mL	40 min
3 × Washing	H_2O dist	3 × 250 mL	3 × 1 min
Developing*)	2.5 % Na_2CO_3 / 0.03% formaldehyde / 0.00075% Na thiosulphate *(6.25 g Na_2CO_3 / 225 µL formaldehyde* / 94 µL Na- thiosulfate (2%))*	250 mL	about 3 min visual control
Stopping	1.4% EDTA-Na *(3 g)*	250 mL	30 min
Preserving	10 % glycerol	250 mL	30 min
Drying	air dry on the support-film		

* add aldehydes shortly before use.

Imaging. Gelcompany ScanFrame (cat# PAS-001-TI) is also useful to prevent curling during the imaging of silver-stained gels.

9. Blotting and Blot Staining of Flatbed Gels

For electrophoretic transfer of proteins on a blotting membrane the film-backing must be removed from the gel. The easiest and safest way to remove the film-backing without damaging the gel layer is cutting with a thin steel wire. This can—for instance—be performed with the help of a 2 ½ L bottle, self-adhesive tape, and a steel wire with two handles at the ends. However the best way is using the FilmRemover as described here:

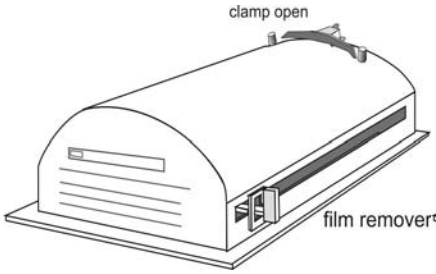


Fig. 9.1: GE Film remover before loading the gel.

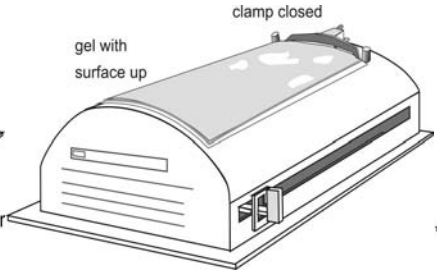


Fig. 9.2: After pre-equilibration with anodal buffer clamp the gel with the film-backing down.

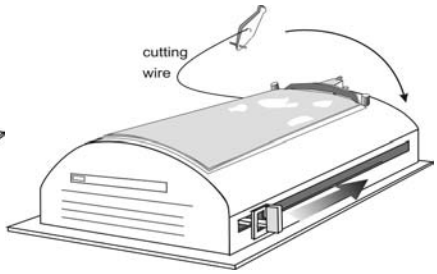


Fig. 9.3: Move the handles forward. grip the tension lever with the cutting wire and hitch it to the right handle.

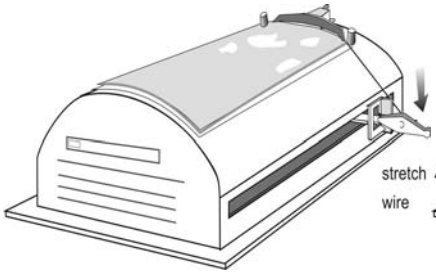


Fig. 9.4: Press down the tension lever to lock and tension the wire.

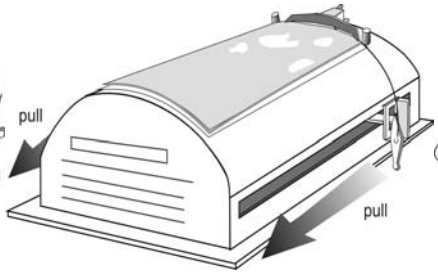


Fig. 9.5: Grip the two handles and pull the wire between gel and film-backing

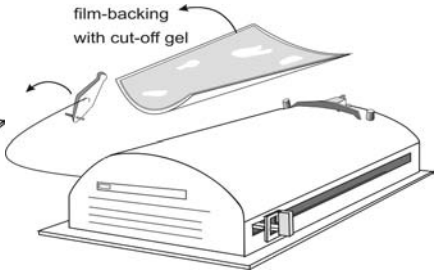


Fig. 9.6: Remove the tension lever with the cutting wire and take off the gel with the film-backing.

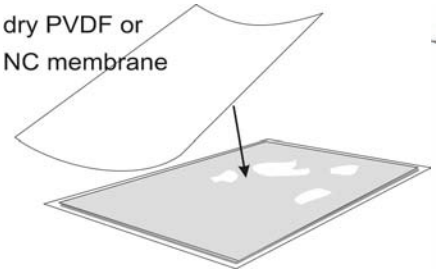


Fig. 9.7: Place film-backing and gel on a flat bench and apply a dry blotting membrane to the gel. Do not try to correct any fold of the gel at this stage as you will damage the membrane

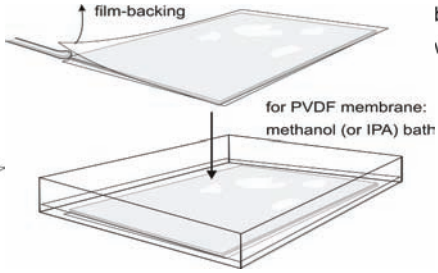


Fig. 9.8: Flip the gel so the backing is uppermost and remove the plastic-backing from one corner, holding the gel corner down with a spatula. For PVDF membranes immerse membrane and gel in methanol (or isopropanol) for a minute and smooth out any folds.

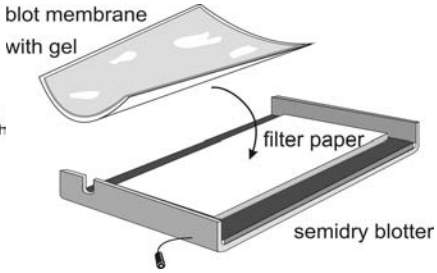


Fig. 9.9: Grip blot membrane and gel at two corners and place them on the filter paper stack.

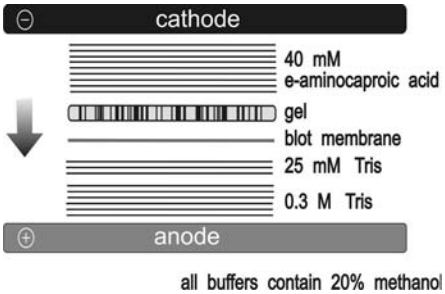


Fig. 9.10: Example for a setup for semidry blotting with a discontinuous buffer system*).

Other blotting transfer systems can be used, for example, tank blotting or semidry blotting with a continuous buffer system. However, the discontinuous buffer system described here is the most efficient (1).

Methanol can be replaced by isopropanol (IPA).

(1) Tovey ER, Baldo BA. Comparison of semi-dry and conventional tank-buffer electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes. Electrophoresis. 8 (1987)

Semi-dry blotting with a discontinuous buffer system

Transfer buffers

Anode solution I:

0.3 mol/L Tris	36.3 g
20% (v/v) methanol*)	200 mL

make up to 1 L with distilled water.

Anode solution II:

25 mmol/L Tris	3.03 g,
20 %(v/v) methanol*)	200 mL

make up to 1 L with distilled water.

Cathode solution:

40 mmol/L ε-aminocaproic acid	5.2g
0.01% (w/v) SDS	0.1 g
20% (v/v) methanol*)	200 mL

make up to 1L with distilled water.

*) Methanol can be replaced by isopropanol (IPA).

Blotting procedure with discontinuous buffer in a semidry blotter

1. For best results run the buffer front off the gel during electrophoresis prior to transfer
2. Wet the graphite anode plate (the plate with the red cable) with distilled water, remove the excess water with tissue paper.
3. Cut the necessary filter papers (6 for the anode I, 3 for anode II, 9 for the cathode) and the blotting membrane to the size of the gel.
4. Slowly soak 6 filter papers in anode buffer I, and place the stack in the middle of the blotting plate.
5. Slowly soak 3 filter papers in anode buffer II and place the stack on top of the first stack.
6. Remove the gel from the film-backing (see previous page) and place it together with blot membrane on the stack.
7. Slowly soak 9 filter papers in cathode buffer and apply the stack onto the gel.
8. Apply the cathodal electrode plate and start the electro-transfer (no cooling):

Example: Apply 200 mA for a Flatbed gel 25 x 12.5 cm

current	*voltage*	**time**
0.8 mA / cm^2	*10 V*	**1 h**

Staining of the blot membranes

LavaPurple

LavaPurple provides the most sensitive method for the staining of protein blots which, due to its reversible binding mechanism, it is fully compatible with downstream processing such as Mass Spec. immuno– or functional– staining. Full details of blot staining with LavaPurple along with other useful information on blotting can be found at in section 6 of this manual and at www.gelcompany.com.

Solutions and Buffers

Solution 1 (fixation and acidification): Place 850mL of high purity water into a 1L bottle then add the contents of packet 'A', and mix until dissolved. Add 150mL of 100% ethanol and mix thoroughly.

Solution 2 (staining buffer): To prepare the staining buffer add 1L of high purity water to a 1L bottle and add the contents of packet 'B'. Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) then add packet 'C' and continue stirring until fully dissolved.

Solution 3 (washing): Mix 850mL of high purity water and 150mL of 100% ethanol in a 1L bottle.

Storage of solutions

Solutions 1-3 can be stored at room temperature and are stable for up to 6-months.

Washing: Following transfer, place the wet membrane in water and wash for 3 × 5min. For small-sized blots use 50mL volumes for all steps. For large-sized blots use 200mL volumes.
Basification: Wash the blot in solution 2 for 10 minutes.
Staining: For small blots add 250µL LavaPurple to 50mL of high purity water. For large blots add 1mL of LavaPurple to 200mL of high purity water. Stain in LavaPurple for 15–30min. Blots should be placed 'protein side' down in the prepared stain.

Follow either the PVDF or nitrocellulose protocol from this point.

PVDF

Acidification: Place the blot in Solution 1 and rock gently for 5min. For large blots use 400mL. For small blots use 50mL. This treatment will cause the blot to appear green.
Washing: Rinse blot with 100% methanol for 2–3min until green background on blot has been completely removed. Multiple rinses may be required.
Drying: Dry for 2–3min. To allow simultaneous drying of the blot on both sides it may be best to dry the blot on a wire mesh. Allow the blot to completely dry. The blot is then ready for imaging and further analysis.

Nitrocellulose

Washing: Place the blot in Solution 2 and rock gently for 5min. Remove from Solution 2 and place into high purity water and rock gently for 5min. Repeat the water washing.

Allow blot to completely dry. Your blot is now ready for imaging and further analysis

Care should be taken to ensure that the membrane does not dry during the staining.

Fast Green staining

Dissolve 0.1% (w/v) of Fast Green in 1% acetic acid;
Stain for 5 min; de-stain the background with distilled water for 5 min;
complete de-staining of the bands is achieved by incubating the film for 5 min in 0.2 mol/L NaOH.

Ponceau S Staining

Dissolve 0.1%(w/v) Ponceau S in 1 %(v/v) acetic acid;

Stain for 5 min;

Destain two times for 5 minutes with 5 % acetic acid.

10. Pattern Evaluation and Image Analysis

There are two types of software used in image analysis.

- Algorithms of first generation detecting spots on all images independently, followed by warping and matching before going to final results.
- Algorithms of second generation aligning images prior to any spot detection, followed by spot detection across series of gels followed by statistics and final results.

In both cases, the quality of the gel image in terms of reproducibility and resolution is one of the most important factor that guarantee correct final results. Recent reproducibility tests made on behalf of HUPO (www.fixingproteomics.org) showed that the reproducibility of high quality standardised electrophoresis gels was much higher that first expected. It is common to see more than 90% of the differential spots common to all labs using second generation algorithms as described here above.

The combination of the HPE Flatbed Tower and HPE gels is ideal for high quality proteomics analysis where true differences induced by the disease or the treatment must be extracted from technical noise and biological variations.
Figure 10.1 shows the perfect auto-alignment obtained on Second generation software algorithms (SameSpots Nonlinear Dynamics).

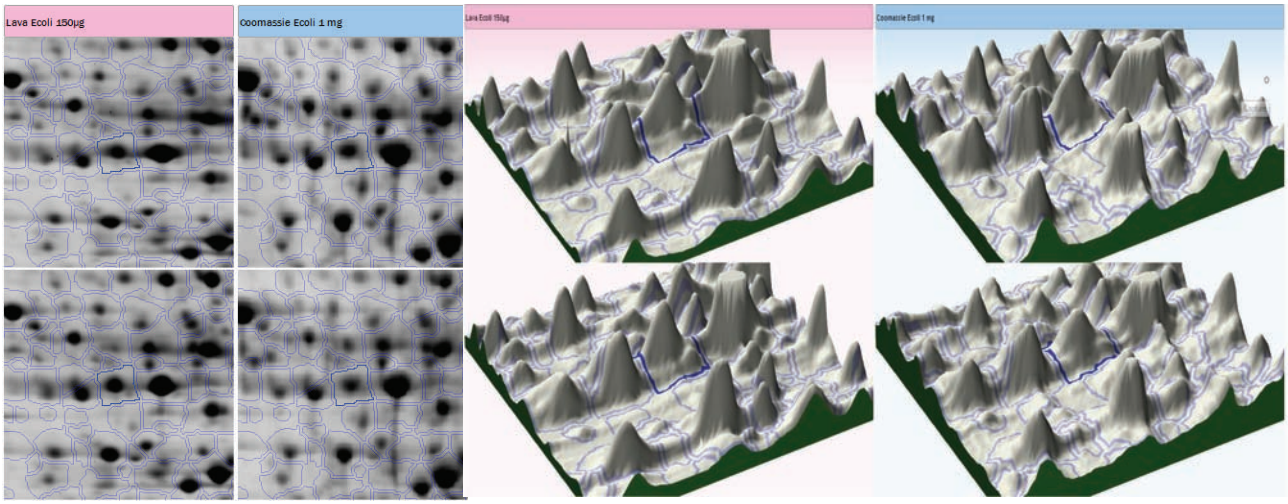


Fig. 10.1: SameSpots Auto-alignment of 2 Lava Purple stained gels with 50 µg *E. coli* and 2 Coomassie stained gels loaded with 1 mg *E. coli.* The 4 gels were HPE Flatbed NF 10-15% run together on HPE Flatbed Tower

Such a high alignment quality is a must in Proteomics. This can only be obtained by combining, reproducible sample preparation, reproducible gels, standardised first and second dimension migration and to finish, the use of a software algorithm that do not create additional variations like a variable number of spots between gels.

11. Band and Spot Picking

Even with after sophisticated detection and image analysis of protein spots the identity of a spot must be proven or its structure must be characterised with mass spectrometry. After electrophoresis and protein detection the bands or spots are therefore cut out from the gel and submitted to tryptic digestion. The peptides are eluted and further analysed with mass spectrometry.
When proteins have been labelled with a fluorescent tag or been post-stained with a fluorescent staining method, they are not directly visible. There are two ways to pick protein bands or spots from the gel: either using a spot-picking robot or post-stain with a visible dye for manual spot-picking. Alternatively, printed gel images can be used to guide manual spot picking. HPE gels are particularly well suited to spot cutting techniques because the gel is mechanically stabilied by the plastic backing.

10.1 Automatic spot picking

Robotic spot pickers are reliable for excising selected spots from the gel slab and transferring them to defined wells of microtiter plates. It can be applied for both: fluorescent visualized spots as well as for spots detected with a visible dye. There are two concepts for automated picking: using a CCD camera for controlling the picking head, or by transferring the pixel coordinates from image analysis into the machine coordinates of the picker instrument for fully automated picking. The latter method is superior, because cameras in pickers offer less sensitivity of detection than scanners.
HPE backed gels are ideal for automatic spot picking, because the gel is immobilised on a rigid support . Two self-adhesive reference markers, glued onto the support plate or film, are necessary to enable the machine to recalculate the x / y positions of the proteins from the imported picking list. The two markers are automatically recognised by the camera and used as calculation points for the spot positions. This procedure provides a very high picking efficiency and accuracy, because it utilises the high resolution and sensitivity of the scanning device. Furthermore, using the image analysis data, also fluorescent, non-stained and radioactive labelled spots can be excised. The camera in this system has only the function to find the positions of the two reference markers.

Fig. 11.1: picking head of an automatic spot picker

10.2 Manual spot picking

Not all laboratories own a robotic spot picker. Sometimes only a few spots need to be picked, which does not justify the set up time for sophisticated system. Then manual picking is the most straightforward procedure.
To facilitate manual spot cutting HPE gels can be post-stained with Coomasssie Brilliant Blue and/or with silver staining (see methods in chapter 8) alternatively printed images can be used to facilitate spot picking (see http://www.gelcompany.com/index.php?option=com_docman&task=doc_download&gid=47&Itemid=80)

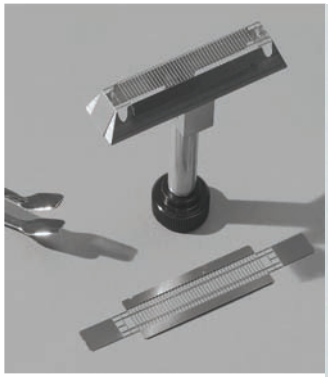


Fig. 11.2: Grid cutter for non-stained bands

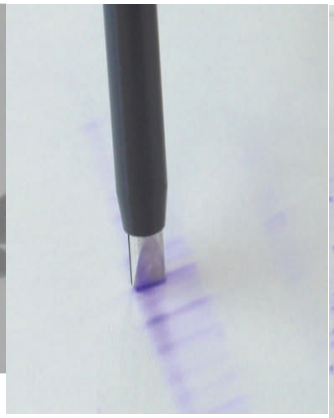


Fig. 11.3: One-touch band picker

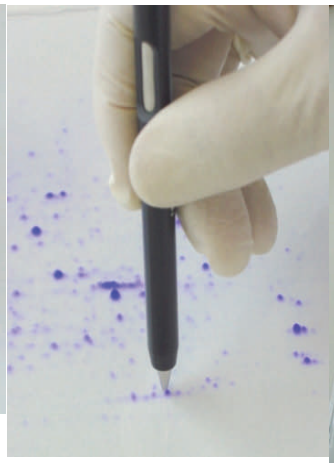


Fig. 11.4: One-touch spot picker

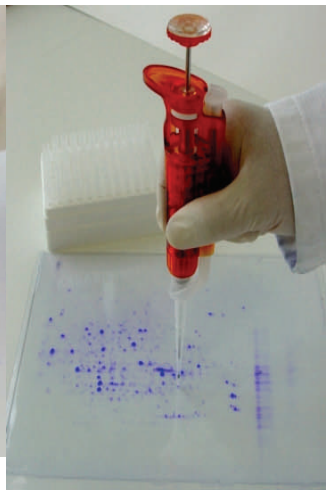


Fig. 11.5: One-touch plus spot picker: Modified pipetter with plunger with floating filter tip to prevent contamination
(US. patent No. 6,702,990).

12. Trouble shooting

Symptom	Cause	Remedy
12.1 Effects during electrophoresis		
Green light on cool water control is not spinning, but static red after set to “Cooling“	Tubing connection to the chiller is wrongly connected or kinked.	Check tubing: Tubing leading from “Out“ of chiller must be connected to “In“ on Tower.
Air bubbles between film-backing and cooling plate.	Volume of cool contact fluid was not sufficient (amount fluid required depends on ambient temperature).	Lift up gel on one side and apply a higher volume
Excess cooling fluid around the film support.	Too much cooling fluid applied on the cooling plate (amount fluid required is depends on ambient temperature).	Remove excess fluid with lint-free tissue paper. Apply smaller volume in future.
Water droplets on the gel surface.	Gel is pre-cooled without lid at high humidity conditions leading to water condensation.	Set cool water flow to “*Bypass*“ while setting up until the lid has been applied then switch cool water flow to “*Cooling*“
No electric current, drawer control lamps stay dark after starting the power supply.	Electronic control detects wrong orientation of electric field.	Plug power supply cables in correctly: black cable to cathode, red cable to anode.
	Electronic control detects that one or more draws do not contain a gel.	When less than four gels are run, unplug the non-used electrode lids and place them into the parking position.
	Lids not properly postioned or not plugged-in.	Re-position lides and check connections between lids and drawers.
Condensation inside of electrode lid.	The gel gets hot during electrophoresis because of insufficient heat dissipation.	Switch cool water from “*Bypass*“ to “Cooling“ when starting electrophoresis
		Check chiller temperature and ensure no other aparatus is connected to the same chiller.
Front is curved instead of straight .	The gel gets hot during electrophoresis because of insufficient heat dissipation.	See above
	FlatTop Tower is subject to hot exhaust from chiller or other apparatus.	Relocate chiller or other apparatus.
	The gel gets hot during electrophoresis because too much power is appied per gel	If you run less than four gels at a time, reduce the mA and W settings in the power supply accordingly. Follow strictly the manual .
Migration of front is very slow and will not reach the anode in time.	The electric field is too low.	
Front is slanted, not straight.	Uneven buffer concentration within the electrode wicks.	Always hold electrode wicks horizontal when carrying them to the gel.
Audible alarms and lamps flashes orange or red.	Not all gels and wicks are connected, leading to differences in current beteen the gels.	See section 1 of manual
Condensation water develops inside electrode lid near to IPG strip(s)	Local heat production at IPG strip(s) because of electroendosmotic effect	Remove IPG strip(s) from gel after the first 70 minutes and then continue the run. Follow strictly the manual.

Symptom	Cause	Remedy
Minor disturbance(s) in the front.	Buffer drop(s) fell on the gel surface	Avoid passing wicks over gel surface.
	Air in between wick individual wicks	Gently roll wicks in gelcompany Paperpool to remove air.
Irregular bulging of the front on one side	Equilibration buffer unequally distributed within the IPG strip(s).	Hold the IPG strip(s) horizontal, start in the middle when placing the strip into the slot.
Run stops, front does not continue to migrate, sparking at the IPG strip(s) .	Strong electroendosmosis effect at the IPG strip(s) , because it has not been removed after the first 70 minutes.	Remove IPG strip(s) from gel after the first 70 minutes and then continue the run. Follow strictly the manual.
11.2 Effects during staining		
Semicolloidal Coomassie blue staining		
Gel starts to curl.	Staining solution too hot for NF film.	Do not heat the staining solution to more than 40 °C.
Small dye particles on the gel surface.	Solid unsoluble material from the dye tablet.	Can be wiped off. Always place the gel with it's gel-side down on the grid of the staininig tray.
LavaPurple staining:		
Dye turns from light purple to light yellow	Staining solution too acidic.	Apply the two pre-buffering steps proposed for film-backed gels. Follow strictly the manual for film-backed gels.
12.3 Effects during scanning		
Gel edges curl up during scanning.	Gel edges start to dry out.	Apply Gel Scan-Frame on the edges of the gel during scanning.
12.4 Effects seen in the result		
Horizontal streaking	The first dimension IEF separation in the IPG strip did not work well because of inappropriate sample preparation IEF separation problems.	Check the trouble shooting guides supplied by the providers of the IPG strips and web forums.
Vertical streaking	Insufficient equilibration of the IPG strip.	Use the equilibration buffer supplied with the HPE gels, weigh-out the correct amounts of DTT and IAA (should be of highest reagent quality), follow the manual.
LavaPurple staining: Unsufficient sensitivity of staining	Wrong protocol for non-backed gels has been applied, or the solution volumes were too small	Follow the protocol of this FlatTop Tower manual which has been optimised for the HPE Flatbed gels.

Legal Information

Gelcompany Pty Limited,
Unit 3 43 – 51 College Street,
. Gladesville, NSW 2111, Australia.

Ordering Information

		Quantity	*gelcompany code no.*
Equipment:			
HPE-FlatTop Tower		1	PPT-001-TI
HPE-Thermostatic Circulator		1	PPC-100-TI
HPE-Power supply		1	PPP-001-TI
Accessories:			
HPE-FlatTop Tower Accessory Kit*			
*Comprising, 4 PaperPools, Scalpel handle, pack of scalpel blades, 2 forceps pointed, 2 forceps blunt, 4 Falcon tubes, scissors and roller		1 kit	PGT-001-TK
PaperPool (Tray for soaking the electrode strips)		4	1003-03
IPG Strip Equilibrator (Tray for IPG strip rehydration and equilibration)		1	1003-04
Steel Tray Standard (with grid, for hot Coomassie staining)		1	1003-25
Steel Tray Large (for large gels, with grid, for hot Coomassie staining)		1	1003-26
Steel Tray Multi 6 (for up to 6 large gels, with 6 grids)		1	1003-27
ScanFrame (for standard and large size gels)		1	PAS-001-TI
Cooling fluid			
HPE-FlatTop Cooling Fluid	50 mL	1	PCC-050-TI
	150 mL	1	PCC-150-TI

Gels and buffer kits (examples only - for other gel types please enquire)

1D SDS Electrophoresis - Standard size (25 x 12.5 cm) gels

Ready to use kits for 1D SDS-electrophoresis including cooling fluid, buffers and electrode wicks

10%*T*, 25 slots for 15µL (4 gels)	1 pack	PCS-125-TK
10%*T*, 52 slots for 6µL (4 gels)	1 pack	PCS-252-TK
15%*T*, 25 slots for 15µL (4 gels)	1 pack	PCS-325-TK
15% *T*, 52 slots for 6µL (4 gels)	1 pack	PCS-452-TK
12.5% *T*, 25 slots for 15µL on non-fluorescent film-backing* (4 gels)	1 pack	PCS-425-TK

*for DIGE-samples and other fluorescent staining

2D Electrophoresis Standard Size (25 x 12.5 cm) gels

HPE-2DGel flatbed NF Kits “Double gels”:

Ready to use kits for running 2 X 11cm IPG-strips (*Double Gels*) including cooling fluid, buffers and electrode wicks.

2DGel flatbed NF 12.5%, 4 gels	1 pack	PCF-902-TK
2DGel flatbed NF 12.5%, 20 gels	1 pack	PCF-202-TK
2DGel flatbed NF 10-15% (gradient gels) 4 gels	1 pack	PCF-903-TK
2DGel flatbed NF 10-15% (gradient gels) 20 gels	1 pack	PCF-203-TK

HPE-2DGel flatbed NF Kits “Triple gels”:

Kits for running 3 X 7 cm IPG-strips (*Triple Gels*) including cooling fluid, buffers, wicks and electrodes.

2DGel flatbed NF 12.5%, 4 gels	1 pack	PCT-301-TK
2DGel flatbed NF 12.5%, 20 gels	1 pack	PCT-302-TK
2DGel flatbed NF 10-15% (gradient gels) 4 gels	1 pack	PCT-321-TK
2DGel flatbed NF 10-15% (gradient gels) 20 gels	1 pack	PCT-322-TK

HPE-*Large* Format 2D Gels 25 X 19.5cm

Kits for running 1 X 24cm IPG-strips (Large Gels) including cooling fluid, buffers and electrode wicks.

2DGel flatbed NF 12.5%, 4 gels	1 pack	PCF-906-TK
2DGel flatbed NF 12.5%, 20 gels	1 pack	PCF-206-TK
2DGel flatbed NF 10-15% (gradient gels) 4 gels	1 pack	PCF-907-TK
2DGel flatbed NF 10-15% (gradient gels) 20 gels	1 pack	PCF-207-TK

Additives:

IPG-Ampholyte Mix, 1mL (40% w/v) for rehydration of IPG-strips.	1 pack	1004-15

Additionally Required (use only highest quality):

Urea, Dithiothreitol (DTT), Iodoacetamide (IAA), SDS marker proteins.

Fluorescent Stain Kit

Kit includes all fixatives and buffers required for fluorescently staining gels:

LavaPurple Protein Kit 100 mL	1 pack	LP-011100
LavaPurple Protein Kit 25 mL	1 pack	LP-011025

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