SERVA Electrophoresis

Heidelberg

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 and thermostatic circulator (chiller)

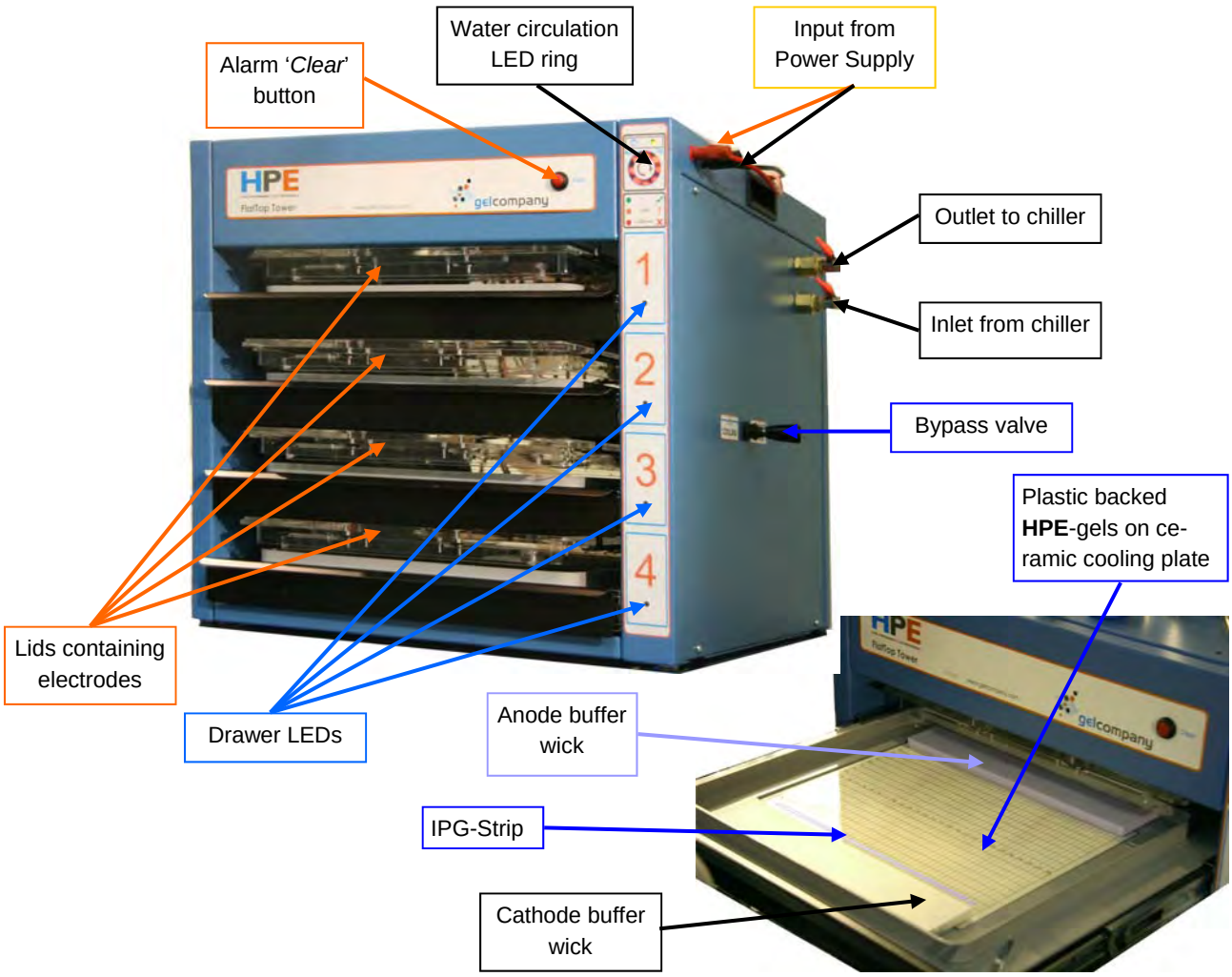
The gels are protected from light during the run, which prevents bleaching of fluorescent labels. No cassettes are required as the plastic-backed gels are run directly on an alumina ceramics cooling plates. The specially formulated **HPE™**-gels allow high voltages to be used in the electrophoresis and when combined with the very thin gels (0.65mm) results in highly focused electrophoretic spots and bands. The specially designed 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 accommodate two different gel sizes. **HPE™**-gels are available in two different sizes:

Standard size: 25 x 11.5 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: 26 x 20.5 cm for:

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

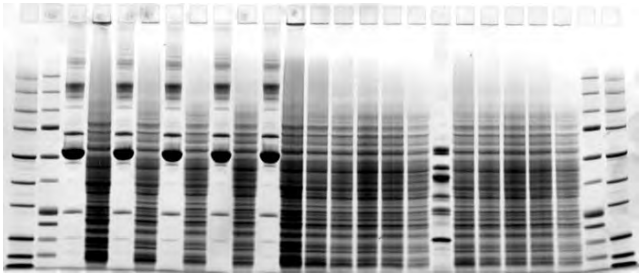


Examples of types of HPE™gels suitable for the HPE™FlatTop Tower

1D SDS PAGE 25 slots

Marker, muscle, *E. coli* extracts

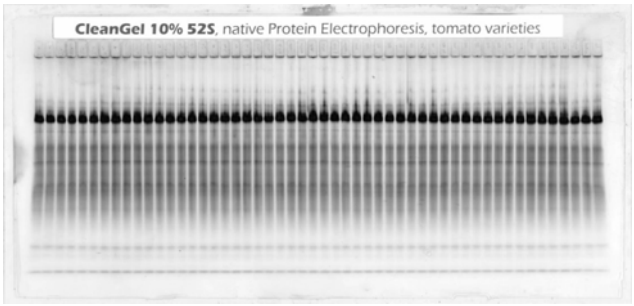
Coomassie® staining



1D native PAGE 52 slots

Tomato seeds

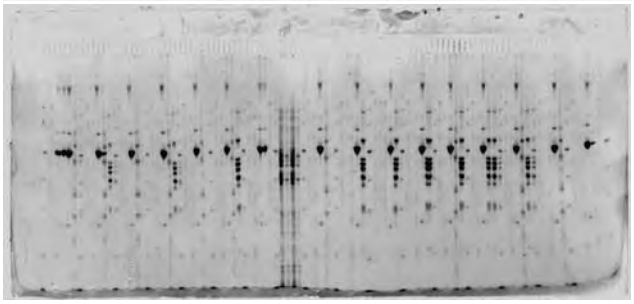
Coomassie® staining



1D SDS PAGE 103 slots

Marker, serum, muscle, seed proteins

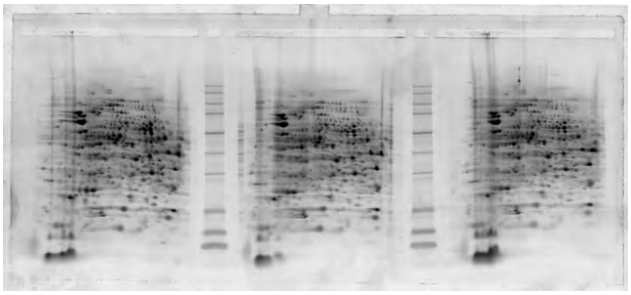
Coomassie® staining



2D SDS PAGE running 3 IPG strips 7 cm "*Triple"* gel

E. coli extracts

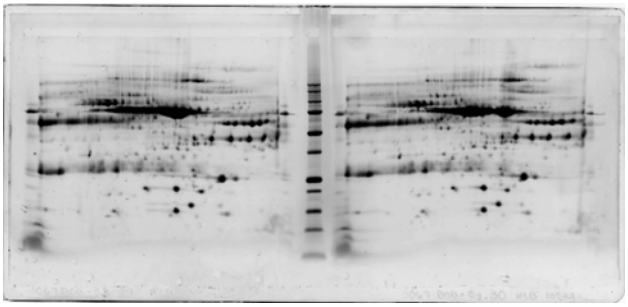
LavaPurple™ staining



2D SDSPAGE running 2 IPG strips 11 cm "*Double*" gels

Human serum proteins

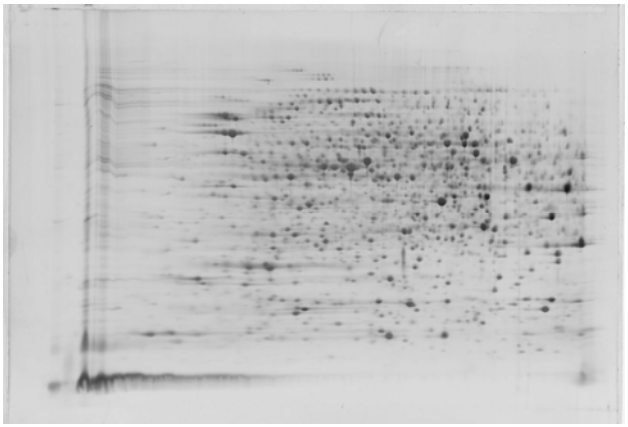
LavaPurple staining



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

E.coli proteins

LavaPurple staining



Coomassie is a Trademark of ICI corporation

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 80KG

- 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 SERVA Electrophoresis GmbH.
- Used in the same state as it was delivered from SERVA Electrophoresis GmbH, 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
- 1 X 1 m thick wall silicone tubing
- Handle for the bypass valve
- This manual.

HPE-FlatTop Tower Accessories comprising:

- 4 PaperPools
- 1 small roller

Please check the contents of your FlatTop Tower package and contact your local SERVA 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 SERVA Electrophoresis GmbH.

If you have any comments on this manual, please send them to us at:
SERVA Electrophoresis GmbH, Carl-Benz-Str. 7, D-69115 Heidelberg, Germany.

1. Assembly, Installation and Running

Unpacking and Assembling of the HPE-FlatTop Tower

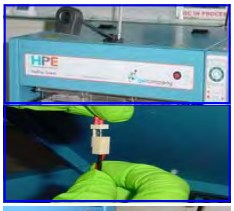
The **HPE**-FlatTop Tower is transported in protective packaging: The base unit and the 4 electrode lids are packed separately and require assembly at the final destination place. A mechanical lifting device and at least 2 people should be used to lift the FlatTop Tower. A ShockWatch label is included 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 **SERVA** representative.

The Base-Unit

Place the base unit on the lab-bench but <u>do not plug-in the power cable</u> 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 <u>do not switch on</u> the FlatTop Tower at this point.

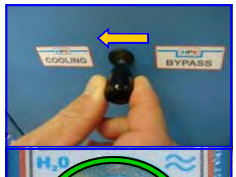


Connecting the Thermostatic circulator (Chiller)

It is important to place the chiller in a position that avoids warm air exhausting from the chiller heating the Flat-Top Tower. In order to achieve optimal cooling effectiveness the chiller must be placed next to the Flat-Top Tower on the same bench level. 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 /"Wait") the power supply.
Opening the drawers with the power supply running causes your power supply to detect a "ground leakage", which results often in a disturbance of the running programme.

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.

Cleaning of the platinum electrodes after each run is particularly important to prevent crystallization of buffer, which can result in uneven contact. Electrodes should be cleaned with a lint-free tissue moistened with distilled water. If the electrodes become tarnished they should be cleaned with a silver cleaning cloth then wiped clean 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 water 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*".

Ensure that the bypass valve is fully open when running gels by pulling it firmly to the cooling position. If the valve is not fully open gels may not be effectively cooled.

Positioning the Gel

With the Flat-Top Tower backed gels are applied directly onto a ceramic cooling plate. To facilitate heat transfer from the gels during electrophoresis, cooling fluid (included in buffer kit) is applied to the surface of the ceramic plate before the gel is applied.

Applying the lids

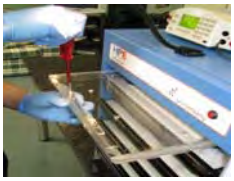
The lids of the FlatTop Tower play an important role as they house the electrodes that contact the buffer wicks. When not in use insert the lids into their "park" positions" below the drawers with the connecting plugs inside the FlatTop Tower.

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 2- 3 minute delay between switching on the power supply and constant illumination of the individual drawer lights. During this 2 - 3 minute delay period the corresponding LED in any drawer where a current is detected will flash green.

Audible alarm alone that beeps every 4 seconds indicates that one or more drawers have been open whilst the external power supply was on. 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. (This alarm has been removed with the new version of the firmware).

Constant illumination of a green LED (after the set-up period) indicates power is supplied to the corresponding drawer and that it is functioning correctly.

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 current to that drawer is >100mA. Power will be shut down to ALL drawers and will not be reactivated until user intervention (drawer opened, power cycled, clear button pushed) 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.

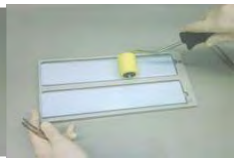
2. Loading and Running Gels

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.serva.de.

Equilibrating IPG-strips


SERVA IPG strip equilibrator 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

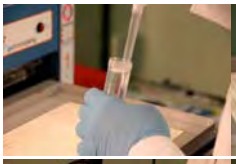



Electrode wicks, soaked in an appropriate buffer, provide a convenient alternative to buffer tanks. The wicks should be fully soaked for at least 15 minutes, thus we recommend you prepare the wicks first.

For large format gels thicker wicks are used to provide sufficient buffer for a run. Air in the wicks can be removed by applying gentle pressure using a roller before the stacks are applied onto the gel. It is practical to soak the anodal 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 SERVA PaperPool. The wicks should be rolled to remove airbubbles and to distribute the buffer evenly. 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

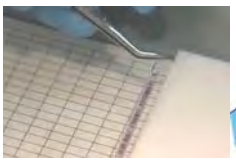
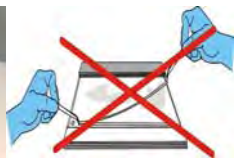




Place the valve is in the "*Bypass"* position to ensure that the gel is not cooled at this stage. To facilitate good contact a specially formulated cooling fluid is added to the surface of the cooling plate. This is then dispersed by sliding a gel from side to side using the gel bent into a "*U-shape*." 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.

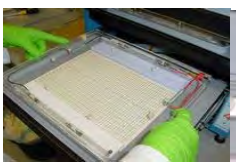
The electrode wicks are then applied with the cathode (white) at the front anode (blue) at the back. The electrode wicks should overlap the gel by at least 2mm. It is important that buffer is not dropped onto the gel surface and you should avoid moving the buffer soaked wicks over the gel. For the anodal wick take the wick around the back of the gel rather than over the top of 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 SERVA . The gels are polymerized on plastic backing, have a size of 25 x 11.5 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). For long shelf-life and optimal separation a Tris-tricine 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 depends 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 Paper-Pool. Add 45 ml of the respective electrode buffer to each wick, distribute the solution evenly with a roller, and allow to soak for at least 10 minutes (Fig 3.1).
3. Apply 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). Lay the gel down on the cooling plate being careful to ensure no air is trapped behind the gel. 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.
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)

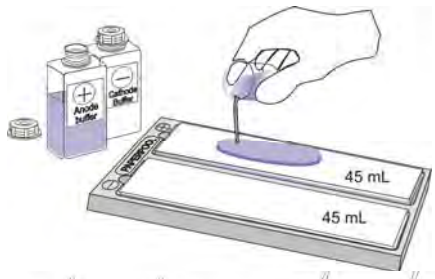


Fig. 3.1

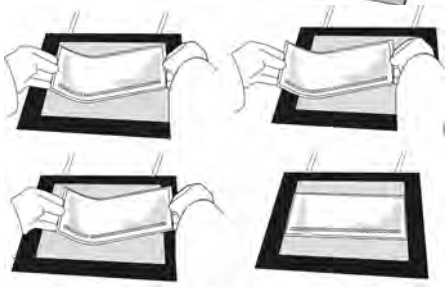


Fig. 3.2

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 ††	50 W †	100 W †	150 W †	200 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 SERVA buffer kit for the running buffers and equilibration solutions.

1. Prepare the two equilibration solutions from the SERVA 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 45 ml of each electrode buffer to the respective electrode wick in the compartments of the SERVA PaperPool (fig. 4.2), distribute the solution evenly with a roller, .

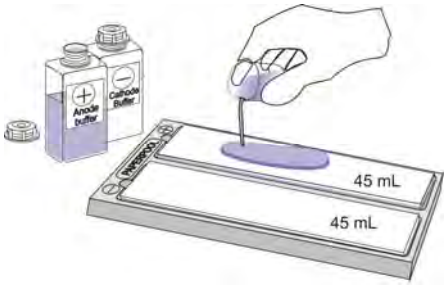


Fig. 4.2

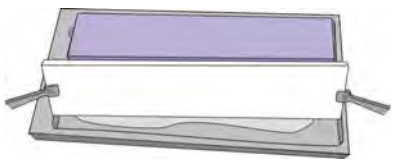


Fig. 4.3

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 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).

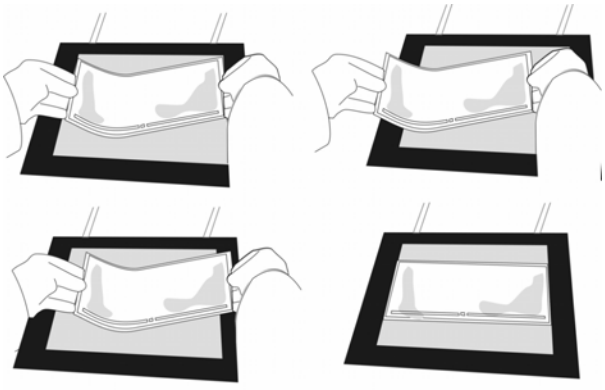


Fig. 4.4

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.

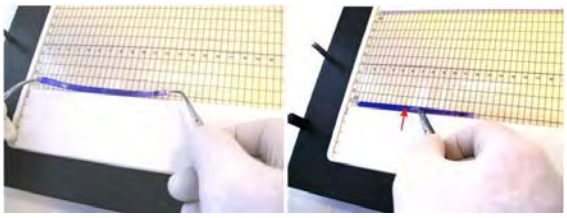


Fig. 4.5

14. Apply 5 µlSDS 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 press "wait" on the powers supply, 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 ††	3 h 00 min*

* valid for homogeneous gels, for the gradient gel 10 -15% this step 4 takes **3.5 h** .

For programming BioRad Power Supplies only:

† - Set as "*constant*"

†† - Set as "*limit*"

5. Two-Dimensional Electrophoresis - Large Gels

Always wear powder free disposable gloves.

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

1. Apply 45 ml of each electrode buffer to the respective electrode wick in the compartments of the SERVA PaperPool (fig. 5.1), distribute the solution evenly with a roller, and soak for a minimum of 15 minutes.

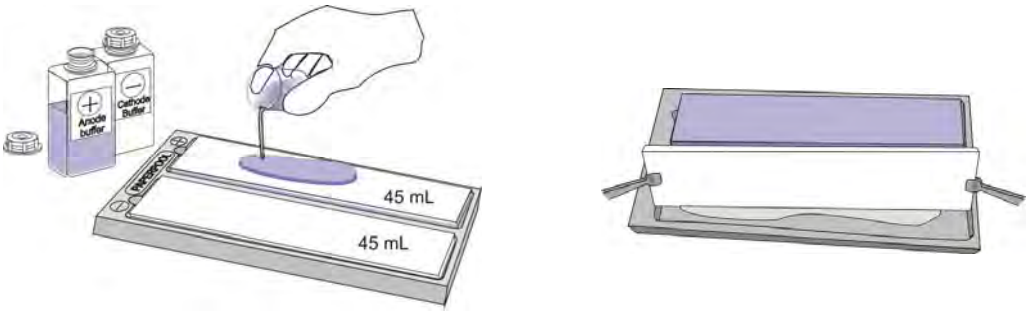


Fig. 5.1: Soaking electrode wicks in 45 ml electrode buffer. .

2. Prepare the two equilibration solutions from the SERVA IPG-Strip equilibration buffer (Eq. buffer).

3. Equilibrate each IPG-strip (gel-side up) in 6 ml solution in a SERVA equilibrator (fig. 5.2) on an orbital shaker with 30 rev/min.

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.

After the 2nd equilibration step discard the solutions.

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

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

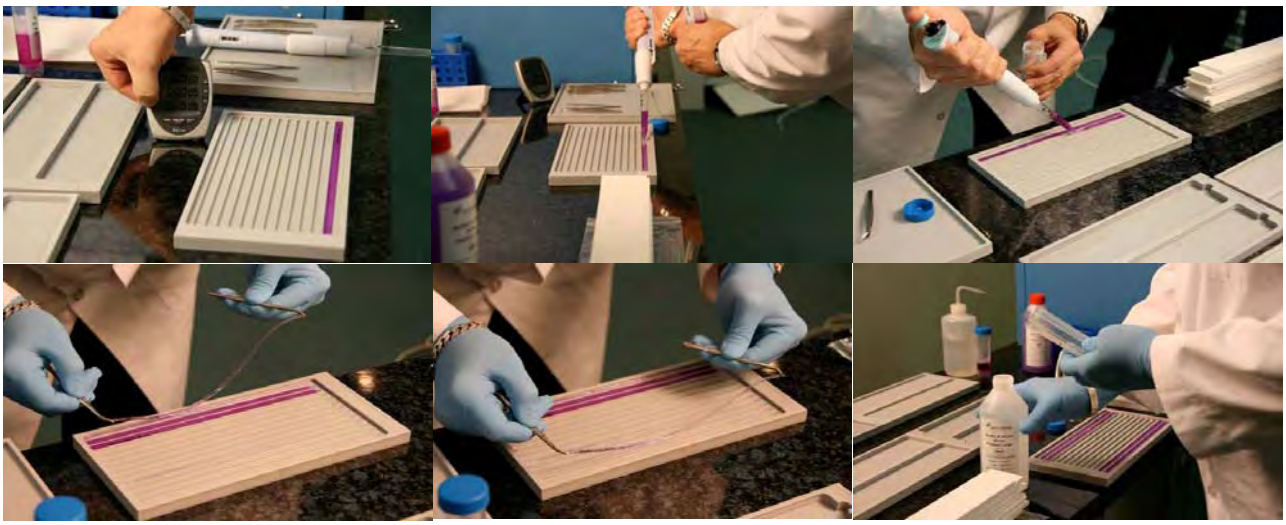


Fig. 5.2: Equilibration of the IPG-strips in 6 ml equilibration buffer.

3. Switch the thermostatic circulator on, set it to 15°C. Switch the FlatTop Tower on, and set the valve to "*Bypass*" to avoid water condensation on the gel surfaces.

4. Apply 4ml of cooling contact fluid onto the center of the cooling plate (fig. 5.3)

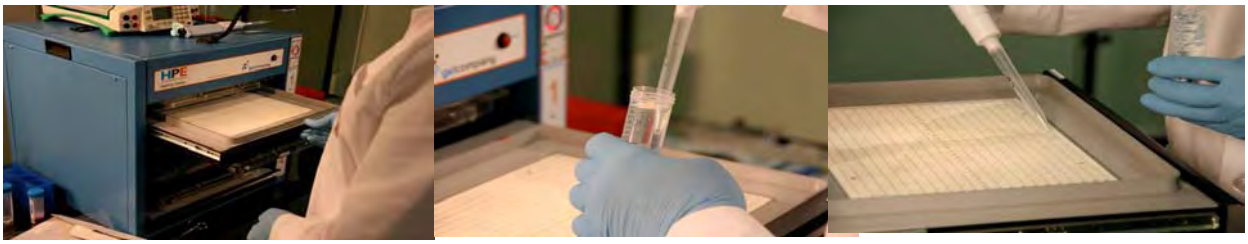


Fig. 5.3: Application of the cooling fluid

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 to the lateral sides of the cooling plate to distribute the cool contact fluid evenly (Fig. 5.4).

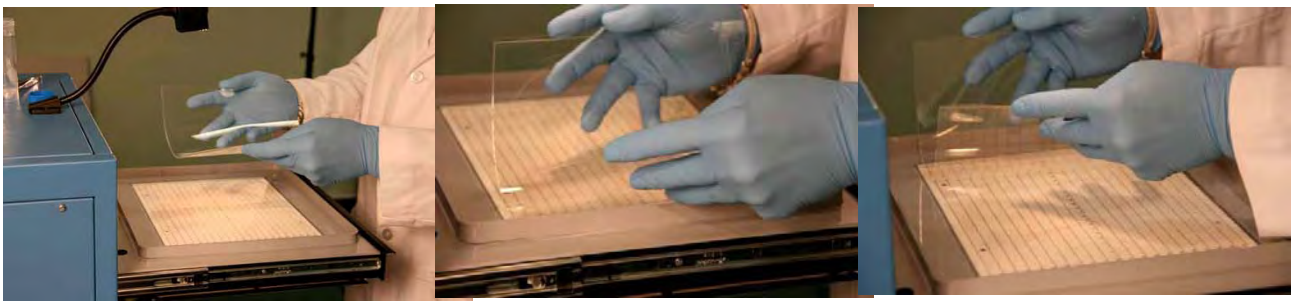


Fig. 5.4: Placing the gel on the cooling plate; distribute the cooling fluid evenly.

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 2-3 mm above the line "*19*" , matching „18.5" (fig. 5.5).

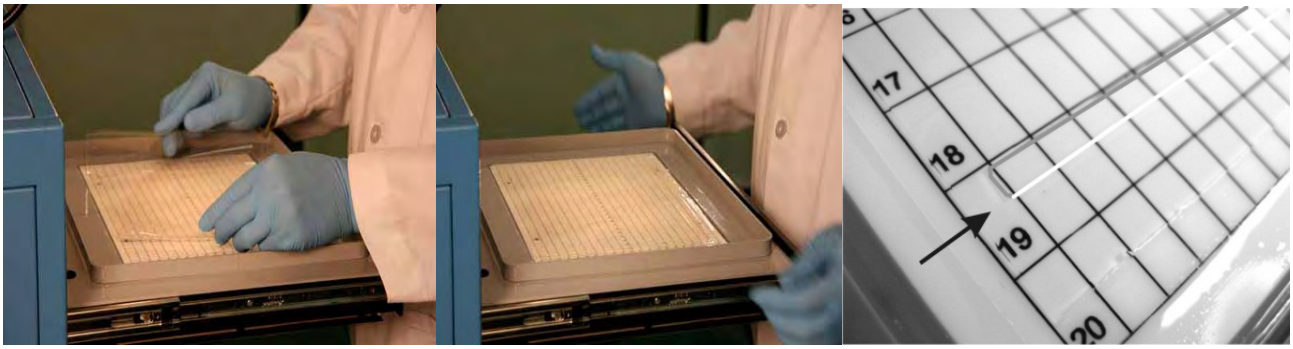


Fig. 5.5: Placing Flatbed *Large* gel on the cooling plate.

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

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

9. Place the electrode wicks onto the gel edges, overlapping the gel by at least 2 mm. Move the anodal wicks around the gel area to avoid dropping buffer on the gel surface (fig. 5.7)

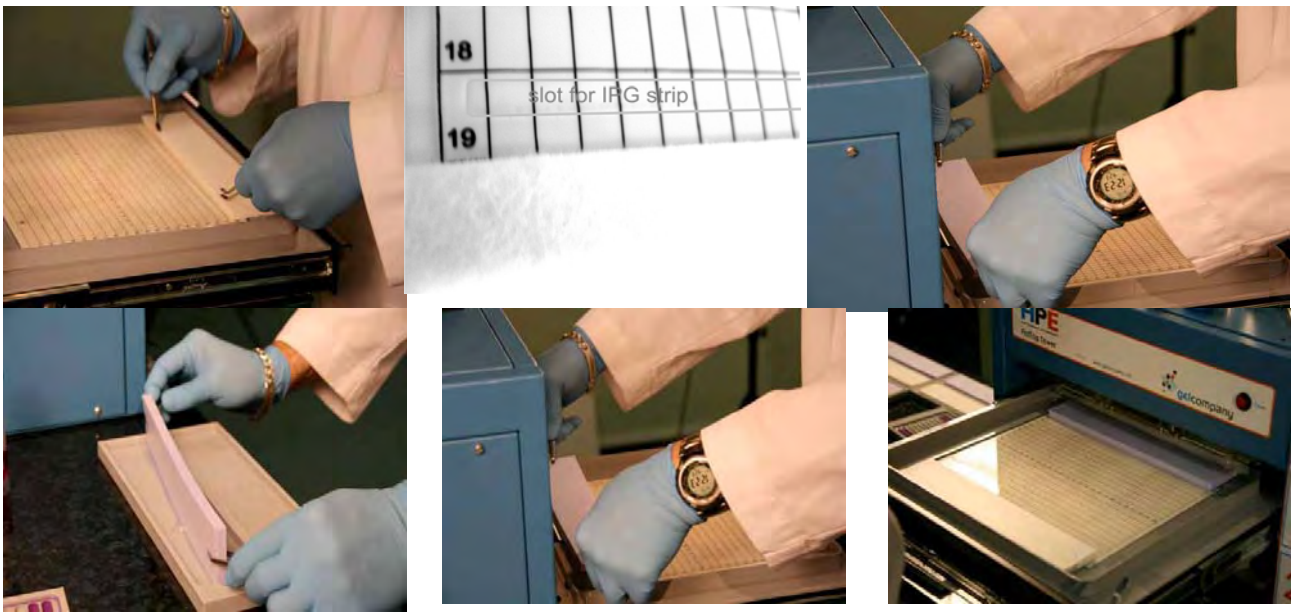


Fig. 5.7: Applying the electrode wicks on gel edges.

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

Important! The IPG strips should not be too dry: Ensure that there is still a thin layer of buffer on the surface.

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 µlSDS marker proteins to the marker well.

13.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*.)

14. After 1 hour 10 min interrupt the run by standby/pausing (press "wait") /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 constant	7mA limit	14mA limit	21mA limit	28 mA limit	1 W limit	2 W limit	3 W limit	4 W limit	30 min
S2	200 V constant	13 mA limit	26 mA limit	39 mA limit	52 mA limit	3 W limit	6 W limit	9 W limit	12 W limit	30 min
S3	300 V limit	20 mA constant	40 mA constant	60 mA constant	80 mA constant	5 W limit	10 W limit	15 W limit	20 W limit	10 min
after this step: **remove the IPG strip.**										
S4	1500 V limit	40 mA constant	80 mA constant	120 mA constant	160 mA constant	30 W limit	60 W limit	90 W limit	120 W limit	3h 50min*
S5	1500 V limit	45 mA constant	90 mA constant	135 mA constant	180 mA constant	40 W limit	80 W limit	120 W limit	160 W limit	50 min

* 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 constant	7mA limit	14mA limit	21mA limit	28 mA limit	1 W limit	2 W limit	3 W limit	4 W limit	30 min
S2	200 V constant	13 mA limit	26 mA limit	39 mA limit	52 mA limit	3 W limit	6 W limit	9 W limit	12 W limit	30 min
S3	300 V limit	20 mA constant	40 mA constant	60 mA constant	80 mA constant	5 W limit	10 W limit	15 W limit	20 W limit	10 min
after this step: **remove the IPG strip.**										
S4	220 V limit	5 mA constant	10 mA constant	15 mA constant	20 mA constant	2 W limit	4 W limit	6 W limit	8 W limit	Hours until 6:00 a.m. next day
S5	1500 V limit	40 mA constant	80 mA constant	120 mA constant	160 mA constant	30 W constant	60 W constant	90 W constant	120 W constant	3 h

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

The specifications "constant" and "limit" are added for programming the Bio-Rad power pack.

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.

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 850 ml of high purity water into a 1L bottle then add the contents of packet 'A' (10 g citric acid), and mix until dissolved. Add 150 ml 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 6.2 g H_3BO_3. Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) then add 3.84 g NaOH and continue stirring until fully dissolved.

Solution 3 (washing): Mix 850 ml of high purity water and 150 ml 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 min to over-night
3. Staining	0.5 % (v/v) LavaPurple in Solution 2	300 ml 1.5 ml Lava-Purple	400 ml 2.0 ml Lava-Purple	1.0 hr
4. Washing	Solution 3 Solution 3 may be re-used up to 2 X	300 ml	400 ml	30 min
5. Acidify	Solution 1	300 ml	400 ml	30 min to over-night

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).

7. Scanning of Backed Gels - Fluorescent

The SERVA Gel ScanFrame 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).

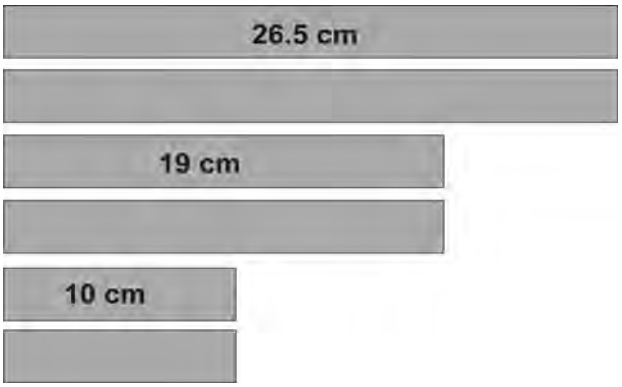


Fig. 7.1: The ScanFrame consist of
two 10 x 25 x 265mm;
two 10 x 25 x 190mm;
two 10 x 25 x 100mm
PVC-bars.

It is suitable of both SERVA 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).

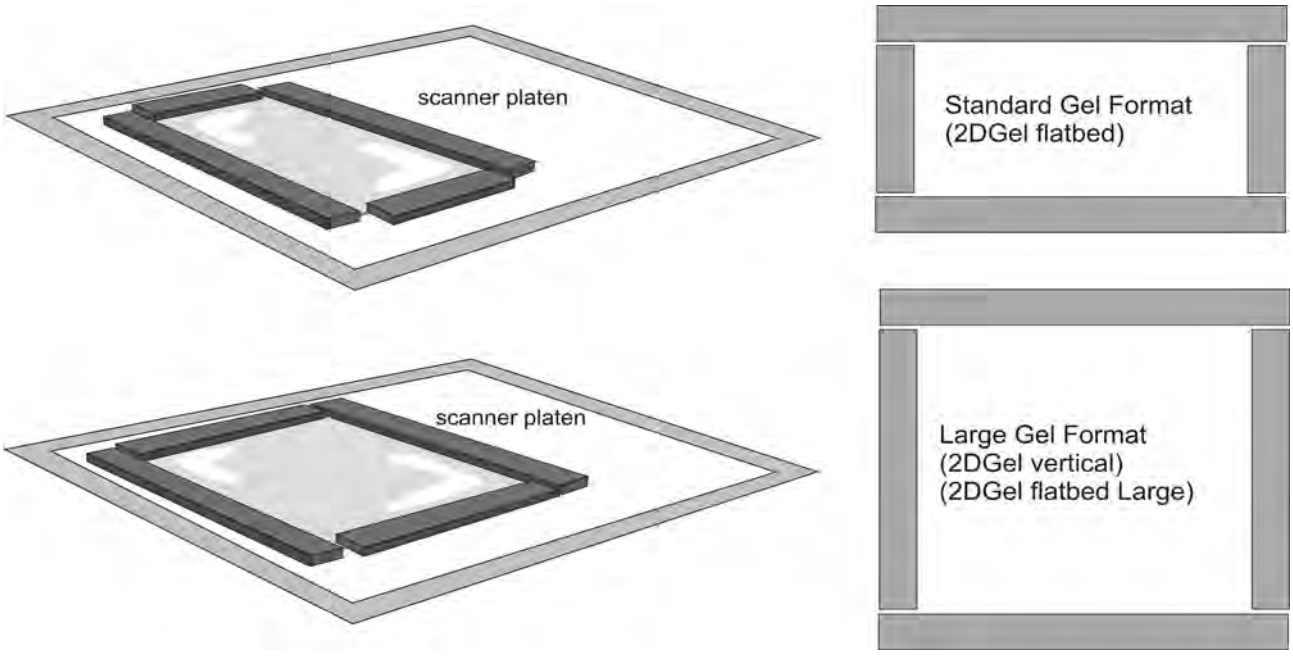


Fig. 7.2: The ScanFrame holding down a normal (25 x 12.5 cm) and a large sized gel (25 x 19.5 cm)

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:

Important: When you scan DIGE gels without fixing and/ or post-staining:
Clean the platen with distilled water and dry it after each scan, to avoid accumulation of air bubbles originating from SDS buffer.

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 - Visible Detection

8.1 Colloidal Coomassie Staining

SERVA HPE Coomassie® Staining Kit (Cat. No. 43396)

SERVA HPE Coomassie Staining Kit is a highly sensitive staining method for 1D and 2D gels after electrophoresis on based on the colloidal Coomassie Blue G®* acc. to Neuhoff et al. (Electrophoresis. 9 (1988) 255-262), that uses dist. water as a destain. All reagents are MS compatible.

1. Fix gels 2 x 1 h with 15 % Ethanol, 1% citric acid.
2. Wash the gel for about 5 min in distilled H_2O before staining.
3. Mix the **dye solution** containing **98 % (v/v) Solution A, 2 % (v/v) Solution B** for several hours. The final **staining solution** contains **80 % (v/v) dye solution and 20 % (v/v) ethanol**. For a large 2D gel, the volume of the staining solution should be at least 300 ml.
4. **Stain** gel with gentle shaking (shaker with ca. 50 – 100 rpm) for at least **3 hours**. Staining over night is optimal. The staining time will be reduced by half when staining is performed at 40 °C to 45 °C.
5. **Destain:** Wash gel after staining in dist. H_2O with regular exchange of the H_2O The band intensity will increase significantly after neutralization in water.

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

SERVA ScanFrame is also useful to prevent curling during the imaging of Coomassie stained gels

8.2 Negative Staining (similar sensitive like Coomassie Staining, fully MS compatible)

SERVA*Snow* Staining Kit (Cat. No. 35080)

Reversible staining method (acc. to Hardy et al. Anal Biochem. 240 (1996) 150-152). Limit of detection: < 10 ng, Fast procedure: about 20 min. Subsequent blotting and elution of proteins is possible.

1. Prepare the working solution I: Dilute Solution I 1:10 with ddH_2O.
2. Prepare the working solution II: Dilute Solution II 1:10 with ddH_2O.
3. Wash the gel with ddH_2O for 30 to 60 s after electrophoresis.
4. Incubate the gel 15 min in **working solution I**.
5. Remove the solution and rinse the gel with ddH_2O.
6. Incubate the gel in **working solution II** until the background turns intense white.
7. Remove the solution and rinse the gel with ddH_2O.
8. Incubate the gel 5 min in washing solution (1:50 dilution of SERVA*Snow* Solution I with ddH_2O).
9. Seal the gel in new washing solution. Store at room temperature.

Drained gels will become transparent. After rehydration staining will be visible again

Destaining and Mobilisation of Proteins for Blotting

Prepare the mobilizing buffer (50 mmol/L EDTA, 25 mmol/L Tris; pH 8.3: 0.76 g Tris + 4.65 g EDTA-Na_2, dissolve in 250 ml distilled water, adjust to pH 8.3 with a few grains of)

Incubate the gel in the mobilizing buffer after staining. The gel will become transparent again and blotting is possible.

8.3 Mass Spectrometry compatible Silver Staining

A number of different Silver Staining methods are available. The two methods below are optimised due for MS-compatibility for staining of backed gels.
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.

8.3.1 Silver Staining Kit

SERVA HPE Silver Staining Kit (Cat. No. 43395)

Sensitivity of detection: about 0.1 ng (BSA)

You can stain the gels also with the following “lab-made” silver staining procedure:

8.3.2 Lab-made Silver Staining

Table 8.1: MS compatible Silver staining of 0.65 mm thin film-backed polyacrylamide gels

step	reagent	volume	time
Fixing 1	10% acetic acid, 15% ethanol, 1% citric acid *(25 ml acetic acid, 100 ml ethanol, 2.5 g citric acid)*	250 ml	30 min
Fixing 2*)	10% acetic acid, 15% ethanol, 1% citric acid *(25 ml acetic acid, 37 ml ethanol, 2.5 g citric acid)*	250 ml	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 mlEtOH)*	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 µlformaldehyde (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 µlNa- thiosulfate (2%))*	250 ml	about 3 min visual control
Stopping	10 % (v/v) acetic acid	250 ml	30 min
Preserving	10 % glycerol	250 ml	30 min
Drying	air dry on the support-film		

*) When staining is done on a staining robot or the same day, the second fixing step should be performed with a solution with the same composition like the Fixing 1 solution. Do not fix over night in 40 % ethanol: this causes strong curling of the film-backed gels.

**)add aldehydes shortly before use.

8.4 Scanning of Visible Stained Gels

Bio-5000 VIS Gel Scanner (Cat. No. SERVA Bio 5000)

White light scanner for transmission and reflection mode. Linearity over 4.0 O.D.

9. Blotting and Blot Staining of Flatbed Gels

SERVA Gel Remover (Cat.-No. HPE-GR01)

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 wire. The best way is using the SERVA GelRemover as described here:

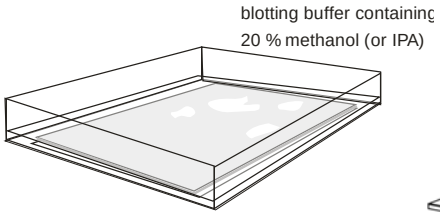


Fig. 9.1: Soak the gel for 10 min in blotting buffer containing methanol to avoid stretching of the gel after removal from film.

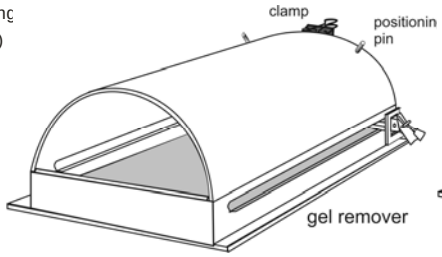


Fig. 9.2: Gel remover before loading the gel.

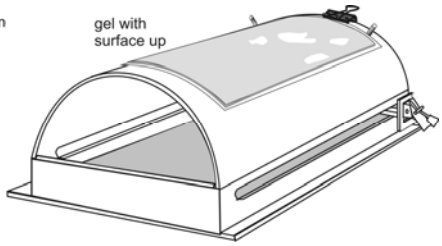


Fig. 9.3: After pre-equilibration with anodal buffer clamp the gel on the gel remover surface with the film-backing down.

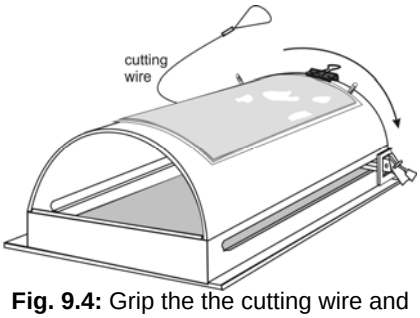


Fig. 9.4: Grip the the cutting wire and hitch it to the right handle.

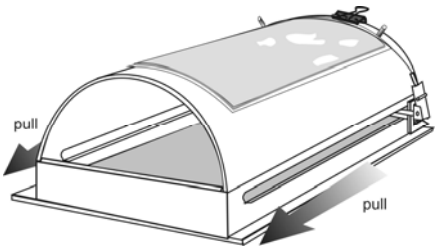


Fig. 9.5: Grip the two handles and pull the wire between gel and film-backing in one smooth movement.

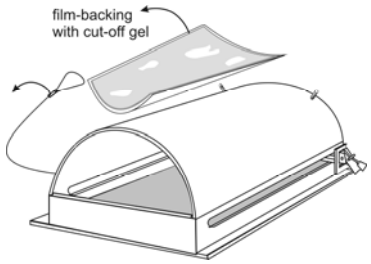


Fig. 9.6: Remove the cutting wire and take off the gel with the film-backing.

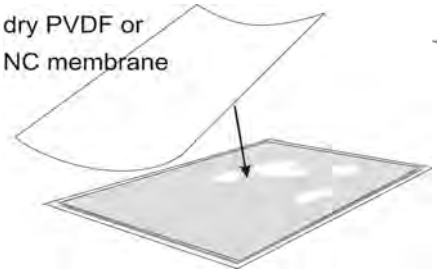


Fig. 9.7: Place film-backing and gel on a flat bench and apply a dry blotting membrane to the gel. Do not try to correct any fold of the gel at this stage as you will damage the membrane

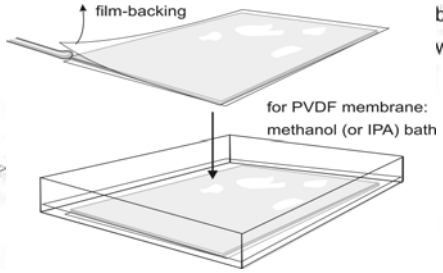


Fig. 9.8: Flip the gel so the backing is uppermost and remove the plastic-backing from one corner, holding the gel corner down with a spatula. For PVDF membranes immerse membrane and gel in methanol (or isopropanol) for a minute and smooth out any folds.

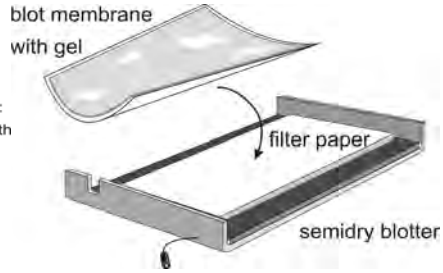


Fig. 9.9: Grip blot membrane and gel at two corners and place them on the filter paper stack.

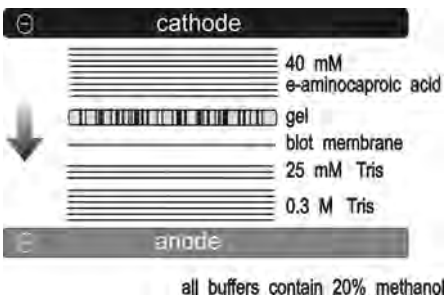


Fig. 9.10: Example for a setup for semidry blotting with a discontinuous buffer system.

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

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.

Solutions and Buffers

Solution 1 (fixation and acidification): Place 850 ml of high purity water into a 1L bottle then add the contents of packet 'A', and mix until dissolved. Add 150 ml 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 6.2 g H_3BO_3 . Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) then add 3.84 g NaOH and continue stirring until fully dissolved.

Solution 3 (washing): Mix 850 ml of high purity water and 150 ml of 100% ethanol in a 1 L 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 × 5 min. For small-sized blots use 50 ml volumes for all steps. For large-sized blots use 200 ml volumes.
Basification: Wash the blot in solution 2 for 10 minutes.
Staining: For small blots add 250 µl LavaPurple to 50 ml of high purity water. For large blots add 1 ml of LavaPurple to 200 ml of high purity water. Stain in LavaPurple for 15–30 min. 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 2–3 min until green background on blot has been completely removed. Multiple rinses may be required.
Drying: Dry for 2–3 min. 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).

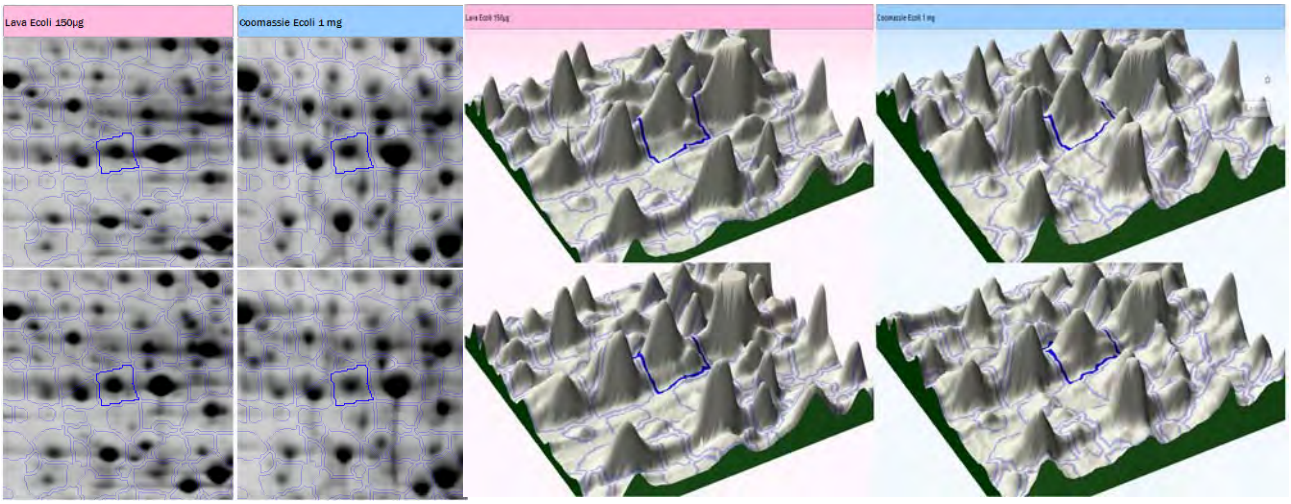


Fig. 10.1: SameSpots Auto-alignment of 2 Lava Purple stained gels with 50 µg *E. coli* and 2 Coomassie stained gels loaded with 1 mg *E. coli.* The 4 gels were HPE Flatbed NF 10-15% run together on HPE Flatbed Tower

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

SERVA HPE™ ScreenPicker (Cat. No. HPE-SP1)

Semi-manual spot picking system

The SERVA HPE™ ScreenPicker is a device to semi-manually pick fluorescent 2D gels (DIGE, LavaPurple, Sypro Ruby, Flamingo…) without the need of post staining and/or sophisticated robot and software. It consists of an embedded screen connected to a small computer to display the scanned image of the gel, an XY carriage to guide the picker accurately over the spot position and a software which can read images and picking lists from most evaluation packages. The ScreenPicker is adapted for gels on 3 mm glass plates (fluorescent markers placed on 3 corners before scanning) and HPE™ gels on non-fluorescent plastic backing (punched holes performed on 3 corners before scanning). The scanned image is projected under the gel. The provided software allows performing a fine-tuning between the size of the displayed image with a mouse and the size of the real gel with the help of markers or punched holes used as landmarks. An XY carriage is used to guide the manual picker over the target to be excised and to prevent parallax errors. The ScreenPicker guides visually the user to place the picked plug in the right well of the microtiter plate using the file generated by the evaluation software. At the end of the process a file containing spot numbers and microtiter plate plug positions is generated for use by digester robot.

Because it clearly marks the spots to pick on the image, the ScreenPicker can also be used for Coomassie® blue or silver stained gels, increasing the picking accuracy and making the process entirely error free.

Another interesting application is the picking of protein spots from 2D gels after the immunological visualization of specific spots by western blotting: The scanned image of the blot membrane is projected under the gel, which contains still a great portion of the proteins after the electro-transfer.

The files from the evaluation software are transferred to the Netbook PC via an USB stick. The special Crunchbang (Debian) Linux installation on the Netbook PC is minimalistic. The Netbook PC setup is not designed for any other purpose than picking spots.

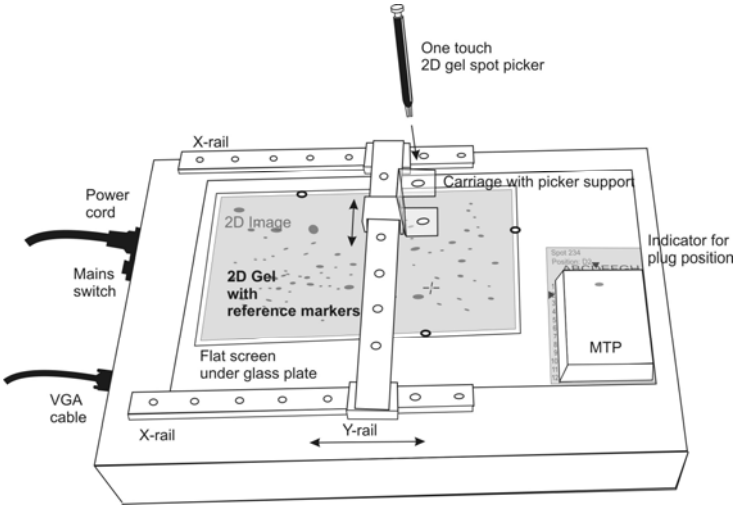


Fig. 11: Schematic drawing of the ScreenPicker box.

Benefits

- Space saving: easy to store on lab bench; dimensions length, width, height compared to huge automatic picker.
- Time saving, accuracy, easy to use, no need for specific training, no need for manual corrections of the gaps always encountered with automatic pickers. Full workflow integration with generation of picking-digester list. Ready within seconds.
- Costs saving: no need for expensive instrument or maintenance contract.
- Error free picking with help of pick list and plate well identifier.
- Flexibility: initialization file defining visual appearance and requirements of digestion robot like input file and reserved positions on plate. Reads any image file format or picking list.
- Safety: no transilluminator or dangerous UV sources.
- Security: Linux avoids transfer of viruses by USB memory keys. PC and Software protected.

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 do not illuminate 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 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		
For all staining methods		
Gel comes off the film support	Too high alcohol concentration for film-backed gels	Reduce ethanol concentration to 15 % (v/v). Film-backed gels do not require high alcohol concentrations for fixing poteins, because the gels cannot swell
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.
	Volume of staining solution too low	Stain gel in the proposed volume.
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.
	IPG strip has become too dry.	Ensure that there is still a thin layer of buffer on the surface.
Local disturbances in the pattern	Air bubble in a buffer wick	Distribute the buffer solutions evenly in the wicks by thoroughly rolling
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 HPE Tower manual which has been optimised for the HPE Flatbed gels.

Legal Information

All goods and services are sold subject to the terms and conditions of sale of the company within gelcompany which supplies them. A copy of these terms and conditions is available on request..

SERVA Electrophoresis GmbH
Carl-Benz-Str. 7
D-69115 Heidelberg
Germany.

Ordering Information

	Quantity	***SERVA** code no.*
Equipment:		
HPE-FlatTop Tower	1	HPE-T01
HPE-Thermostatic Circulator	1	HPE-CU1
HPE-Power supply	1	HPE-PS1
Gel Remover	1	HPE-GR01
Bio-5000 VIS Gel Scanner	1	Bio 5000
HPE-ScreenPicker	1	HPE-SP1
Accessories:		
PaperPool (Tray for soaking the electrode strips)	4	HPE-A02
IPG Strip Equilibrator (Tray for IPG strip rehydration and equilibration)	1	HPE-A04
ScanFrame (for standard and large size gels)	1	HPE-A05

Cooling fluid

Cooling Contact Fluid	50 ml	1	43371.01
	150 ml	1	43370.01

For IPG strips, Gels and Buffer kits see SERVA HPE Catalogue

Additives:

Servalyte® Mix, 2ml (40% w/v) for rehydration of IPG-strips.	1 pack	42940.04
Urea,	1 kg	24524.02
CHAPS	5 g	17038.02
Dithiothreitol (DTT),	5 g	20710.03
Iodoacetamide (IAA)	5 g	26720.01

SDS marker proteins (examples only - for other standards see: www.serva.de)

Dual Color Protein Standard III 7—240 kDa	500µl	39252.01
Recombinant SDS PAGE Protein Marker 10 - 150 KDa PLUS, liquid mix	500µl	39218.01

Staining

LavaPurple™ Fluorescent stain	25 ml	43373.01
	4x 25 ml	43373.02
HPE Silver Staining Kit	1 kit	43395.01
HPE Coomassie® Staining Kit	1 kit	43396.01

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