# omniPAGE Mini Vertical Electrophoresis and Electroblotting Units

## Instruction Manual

**Catalogue Numbers**

CVS10CBS SB10

Record the following for your records:

Model _____

Catalogue No. _____

Date of Delivery _____

Warranty Period _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

# Contents

# Safety Information

When used correctly, these units pose no health risk. However, these units can deliver dangerous levels of electricity and are to be operated only by qualified personnel following the guidelines laid out in this instruction manual. Anyone intending to use this equipment should read the complete manual thoroughly. The unit must never be used without the safety lid correctly in position. The unit should not be used if there is any sign of damage to the external tank or lid.

Acrylamide is a powerful neurotoxin in solution form. Polymerized gels can contain some unpolymerized solution and protective gloves and clothing must be worn.

These units comply with the following European directives:

***2006/95/CE Low Voltage Directive and 2014/30/UE (official Title 2004/108/EC) EMC Electromagnetic Compatibility***

*By virtue of the following harmonised standards:*

***BS EN IEC 61010-1: 2010 Safety Testing of Lab Equipment***

***BS EN IEC 61326-1:2013 EMC Electro Magnetic Compatibility***

# Packing List

Each Units include tank, lid, cables and the following items:

| SKU | Inner inserts | Glass Plates | Combs | Caster | Cooling Pack | Extra |
|---|---|---|---|---|---|---|
| **CVS10CBS** | CVS10DIRM - omniPAGE Mini<br><br>VS10BI - omniBLOT Mini | VS10NG – Notched, Pk/2<br>VS10PGS1 – Plain with bonded 1mm spacers, Pk/2<br>VS10-DP – Dummy Plate | 2 of VS10-12-1 1mm thick, 12 sample | VS10DCAST | VS10ICB | VS10-SCREW x 4<br>CSL-WBROLLER |
| **SB10** | VS10BI - omniBLOT Mini<br>Includes:<br>4 of SB10C Cassettes<br>2 of SB10F, Fibre pads Pk/8 | | | | VS10ICB | CSL-WBROLLER |

**Packing List Checked by: _____**

**Date: _____**

**The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received.**

**Cleaver Scientific is liable for all missing or damaged parts / accessories within 7 days after customers have received this instrument package. Please contact Cleaver Scientific immediately regarding this issue. If no response within such period is received from the customer, Cleaver Scientific will no longer be liable for replacement/damaged parts.**

**Please contact your supplier if there are any problems or missing items.**

# Care and Maintenance

## *Cleaning the Unit*

Units are best cleaned using warm water and a mild detergent. **Water at temperatures above $60^0$ C can cause damage to the unit and components.**

The tank should be thoroughly rinsed with warm water or distilled water to prevent build up of salts but care should be taken not to damage the enclosed electrode and vigorous cleaning is not necessary or advised.

Air drying is preferably before use.

**The units should only be cleaned with the following:**

Warm water with a mild concentration of soap or other mild detergent.

Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons

The units should not be left to in detergents for more than 30 minutes.

**The units should never come into contact with the following cleaning agents, these will cause irreversible and accumulative damage:-**

Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol Alkalis.

## *RNase Decontamination*

This can be performed using the following protocol:-

Clean the units with a mild detergent as described above.

Wash with 3% hydrogen peroxide (H2O2) for 10 minutes.

Rinsed with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,

**Caution:** DEPC is a suspected carcinogen. Always take the necessary precautions when using. RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

# Operating Instructions

Further information (including videos) regarding setting up and running the omniPAGE units can be found at www.cleaverscientific.com

## Usage Guidance and restrictions:

- Maximum altitude 2,000m.
- Temperature range between 4°C and 65°C.
- Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C.
- Not for outdoor Use.

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664.
POLLUTION DEGREE 2, states that: "Normally only non-conductive pollution occurs. Occasionally, however, a temporary conductivity caused by condensation must be expected".

## Setting up the Gel Tanks

**Note: Before setting up the Gel Tank please ensure that it has been properly cleaned and dried.**

1. Note the position of the lid on the unit. This shows the correct polarity and the correct orientation of the cables, black is negative and red positive.
2. Remove the lid from the unit.

**Note: If the lid is not removed, fitting the cables may result in un-tightening of the gold plug and damage the electrode.**

3. Screw the cables into the tapped holes as fully as possible so that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid and the unit is now ready to be used.

*The unit is now ready to be used.*

# Protein electrophoresis

## Casting Unit Preparation

*Cleaning the Glass Plates*

Clean a set of glass plates for each gel first with distilled water and then with 70 % ethanol. One set of glass plates constitutes one notched glass plate and one plain glass plate with bonded spacers.

When using a triple glass plate sandwich, two notched glass plates are required, one set of free spacers and a set of plain glass plates with bonded spacers. The plain glass plate is positioned outermost, then a notched glass plate, free spacers and second notched glass plate. Alternatively, accessory notch glass plates with bonded spacers are available.

**Note: All glass plates, gel casting modules, casting base and accessories must be completely dry before the set – up. Wet components are more likely to miss-align and cause leaks.**

*Gel Cassette Assembly*

Assemble the glass plates in the CVS10DRIM casting/running insert so that the bottom of the glass plates and the spacers are perfectly aligned on a flat levelled surface (i.e. laboratory bench – outside the casting base).

A triple plate sandwich can be used by combining the standard Notched plate and spacer plate, with an additional notched plate with spacers. For triple plate sandwiches, the free spacers Need to be perfectly aligned which is best performed using a small spacer or comb to push the spacers apart. Notched glass plates with bonded spacers do not need manual alignment.

**NOTE: If only one gel needs to be casted, please make sure to use the dummy plate on the other side otherwise the casting/running insert won't be straight.**

*Casting Stand Assembly*

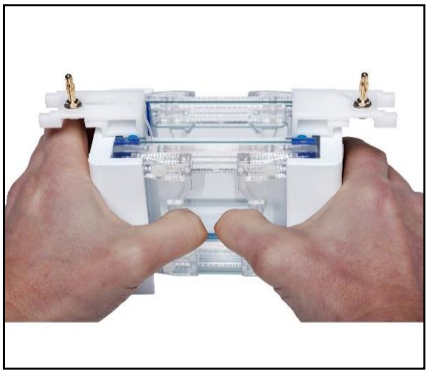
1. Ensure that the pressure bars are adequately open for the thickness of spacer used. The bar can be opened by loosening the screws or by sliding the clamps. When using a triple glass plate sandwich, the

pressure bars will need to be in the completely open position and it is advise to use the screws clamping system instead of the clamps.

2. Position the Slab Gel Insert on a flat surface. Insert the glass plates into the Slab Gel Insert between the pressure bar and the blue gasket. The Slab Gel Insert contains pressure bars which impart even pressure onto the glass plates and allow even screw pressure transfer onto the sealing edge of the glass plate, ensuring complete sealing.
3. Fully tighten the screws, making sure not to wobble the unit. When using the Slide Clamp Mini version, simply slide both gates outwards until fully tightened. When only one gel is being run, the dummy plate must be used in the second position and fully tightened.

**NOTE: At this stage, check that the bottom edges of the spacers and glass plates are perfectly aligned.**

4. Position the Slab Gel Insert in the casting base such that the Cam pins have handles pointing downwards and are located in the insert holes. The top of the module may need to be pushed down very slightly to locate the cam pins by placing a gloved hand horizontally between both glass sandwiches.
5. With the cam pin handles facing directly downwards, turn the cam pins fully through 135° or until the insert has tightened onto the silicone mat.



**NOTE: It is best to turn the cams in opposite directions to each other. Do not overturn as this will cause the glass plates to push upwards and the assembly will be more likely to leak. The unit is now ready for gel preparation and pouring.**

**Always reverse the silicone mat after casting to avoid indentations from persisting. Never leave the casting up-stand with glass plates tightened into the casting base for long periods of time as this will also cause indentations in the silicone mat.**

**The slide clamp version omniPAGE Mini also includes screws. This system can be used either with the slide clamps or screws as preferred by the user. For those that prefer to use the screws rather than clamps, the screws can be simply inserted into the screw holes. The clamps can be removed by placing each clamp in the fully open position and gently bending the clamp upwards from the slanted end. The holding pin will then slowly release, and the clamp can be removed. Screw based casting is required when using the triple sandwich plate method.**

## Gel Preparation

Care should be taken when selecting the pore size of the gel to be used. The pore size or % of gel determines the resolving ability given different sizes of protein.

Gel percentage for various protein sizes are shown below:

| Acrylamide Percentage | Separating Resolution |
|---|---|
| **5 %** | 60 - 220 KD |
| **7.5 %** | 30 - 120 KD |
| **10 %** | 20 - 75 KD |
| **12%** | 17 – 65 KD |
| **15 %** | 15 -45 KD |
| **17.5%** | 12 – 30 KD |

Gel Volumes for the omniPAGE Mini Unit are shown below:

| Number of gels | Gel Thickness (mm) | Volume (ml) |
|---|---|---|
| **Single – one gel, one dummy plate** | 0.5<br>1.0<br>1.5<br>2.0 | 3.8<br>7.5<br>11.3<br>15.0 |
| **Double – two gels** | 0.5<br>1.0<br>1.5<br>2.0 | 7.5<br>15.0<br>22.5<br>30.0 |
| **Using a Triple Plate sandwich – four gels** | 0.5<br>1.0<br>1.5<br>2.0 | 10.0<br>30.0<br>45.0<br>60.0 |

Prepare gel solutions as per tables below. These give the volumes of solutions from the standard stock solutions. These should be gently mixed avoiding generation of bubbles which will inhibit polymerization by removing free radicals.

Resolving Gel, for 2 x 1mm thick gels:

| Solution | 5 % | 7.5% | 10 % | 12% | 15 % | 17.5% |
|---|---|---|---|---|---|---|
| **Distilled Water** | 8.7ml | 7.5ml | 6.3ml | 5.25ml | 3.75ml | 2.5ml |
| **30 % Stock Acrylamide Solution** | 2.5ml | 3.75ml | 5ml | 6ml | 7.5ml | 8.75ml |
| **4 X Resolving Tris Solution** | 3.75ml | 3.75ml | 3.75ml | 3.75ml | 3.75ml | 3.75ml |

Add 15 µl of TEMED and 150µl 10 % Ammonium Persulphate to the resolving gel solution before pouring to initiate polymerisation

Stacking Gel:

| Solution | Volume |
|---|---|
| **Distilled Water** | 4.2ml |
| **30 % Stock Acrylamide Solution** | 0.65ml |
| **4 X Stacking Gel Tris Solution** | 1.6ml |

Add 67µl of 10 % Ammonium Persulphate and 6.7µl TEMED before pouring.

## Preparation of denatured protein samples for loading

The instructions given below are for denatured samples. For Native samples, please consult a laboratory handbook.

1. Prepare the protein samples for loading. The volume of sample depends on the capacity of the wells
2. Using a 0.5 ml micro-centrifuge tube or other convenient receptacle, combine the protein sample and 4 X sample buffer. It is always advisable to use protein markers in one of the end lanes to indicate sizes of bands. These should be prepared according to the manufacturer's instructions.
3. Heat the samples in a water bath or heating block for 2 minutes to denature the samples.
4. Centrifuge the samples in a micro-centrifuge for 20 seconds at 12,000 rpm. The protein samples are now ready to load.

## Gel Pouring

*Casting a gel with stacking layer*

1. Place a comb into the gel cassette assembly with any gel and mark the glass plate below the comb teeth. This is the reference level to which the resolving gel should be poured.
2. Prepare the resolving gel solution. Mix well and avoid generating air bubbles.

3. Fill the glass plates smoothly till the mark avoiding generating any air bubbles. Filling must be performed quickly before the TEMED causes the gel to become too viscous.
4. Overlay the gel extremely carefully with 1 ml of Isobutanol, Isopropanol or distilled water. When using distilled water extra care must be taken to ensure there is no mixing with the gel solution.
5. Let the resolving gel polymerize. Usually this takes around 15 to 30 minutes, but this can vary due to the freshness of the reagents used. If polymerization is taken a lot longer than this, use fresher stock solutions or add more APS and TEMED.
6. Let the resolving gel polymerize. Usually this takes around 15 to 30 minutes, but this can vary due to the freshness of the reagents used. If polymerization is taken a lot longer than this, use fresher stock solutions or add more APS and TEMED.
7. Prepare the stacking gel solution.
8. Before casting the stacking gel, insert a piece of filter paper to dry the area in between the glass plates above the resolving gel. Take care not to touch the surface of the gel.
9. Carefully pour the stacking gel solution, avoiding generating air bubbles.
10. Carefully insert the comb making sure that no air bubbles get trapped under the ends of the comb teeth as these will inhibit sample progression.
11. Allow the stacking gel to polymerize.
12. Once the gel is polymerized it is ready for the electrophoresis run.

*Casting a gel without stacking layer*

- Prepare the resolving gel solution. Mix well and avoid generating air bubbles.
- Pour the solution smoothly into the glass plates avoiding any air bubbles until the top of the notched glass plate is reached.
- Carefully insert the comb making sure that no air bubbles get trapped under the ends of the comb teeth as these will inhibit sample progression.

- Let the gel polymerize. Usually this takes from 15 to 30 minutes, but this can vary due to the freshness of the reagents used. If polymerization is taken a lot longer than this, use fresher stock solutions or add more APS and TEMED.
- Once the gel is polymerized it is ready for the electrophoresis run.

*Using Precast Gels*

1. omniPAGE mini is compatible with all the precast gels available in the market.
2. Simply remove the precast gel from the storage pouch.
3. Gently remove the comb.
4. Keep the Inner module upstand on a flat surface and place the precast gel between the pressure bar and the blue gasket.

omniPAGE tank assembly and Sample loading

1. If desired, fit the cooling pack(s) into the end of the tank. These should be pre-frozen and fitted with the longest side positioned sideways with the end(s) of the tank and pressed into the recess. Or these can be fitted down the front of the tank.

**Note: NEVER FIT THESE UNDERNEATH THE MODULE IN THE BOTTOM OF THE TANK AS THIS WILL PREVENT THE FLOW OF CURRENT THROUGH THE GEL AND CAUSE SLOW RUNS AND OVER-HEATING.**

**Note one pack is supplied as standard. Additional packs can be purchased.**

2. Transfer the Inner gel module containing cast gels into the main tank in the correct orientation as indicated - +ve on the module aligned with +ve on the tank, -ve on the module aligned with –ve on the tank.
3. Fill the outer tank with 1X reservoir buffer. See "Solutions" for recommended running buffer solution.
4. Load the samples into the wells using a pipette tip taking care not to damage the wells or induce any air bubbles.
5. Fill any unused wells with 1 X sample buffer.
6. It is a good idea to note the orientation and order the samples were loaded in. This can be done by noting which samples were loaded adjacent to each electrode.

| **Buffer Volume** | **omniPAGE mini vertical** |
|---|---|
| **Minimum** – Inner tank is filled to above gel level.<br>Outer Tank is filled to just flood the bottom of the glass plates. Cooling potential is at a minimum which may affect resolution. | 250ml |
| **Maximum** – Inner tank is filled to above gel level.<br>Outer Tank is filled to the maximum fill line. Cooling is high offering good resolution of samples. | 1200ml |
| **Using the cooling packs** – Inner tank is filled to above gel level.<br>Cooling packs are inserted behind the gels. Outer Tank is filled to the maximum fill line. Cooling is at a maximum. | 1000ml |

## Gel Running

1. Fit the lid and connect to a power supply.
2. Gels should be run at constant voltage, with voltage dependent on the size of the proteins undergoing separation. An initial setting of 100V with constant observation of migration and heat production is recommended. An exploratory study may be required to determine the optimal settings for individual proteins.

| **Suggested Voltages and Resultant Current for 1mm thick, 12% gels.** | **omniPAGE mini vertical** |
|---|---|
| One gel | 90-225V<br>20-45mA |
| Two gels | 90-225V |

| | |
|---|---|
| | 40-90mA |
| Three gels | 90-225V<br>60-135mA |
| Four gels | 90-225V<br>80-180mA |

## Gel Removal

1. Turn the power supply off when the loading dye reaches the bottom of the gel, sooner if your proteins are below 4 kDa in size.
2. Remove the gel running module, first emptying the inner buffer into the main tank. Buffer can be re-used but this may affect run quality if continued.
3. Unscrew the glass plates with the Screw version. To open the sliding door version, insert the CSLKEY into the recess arch of the clamping door. Twist key applying pressure to both the clamping door and the CVS10D side cheek. The door will now click open. Repeat this process until you have opened both the doors.
4. Remove the glass plates. Then using CSLKEY separate notched and the plain glass plates. Place the wedged end of the key between the two plates and gently twist until the plates pull apart. The gel will usually stick to one of the plates and can be removed by first soaking in buffer and then gently lifting with a spatula.
5. The gel is now ready to be stained with Coomassie or silver stain or the proteins in the gel can be transferred to a membrane by electroblotting for specific band identification and further analysis.

# Protein Blotting

## *Setting up the blot sandwich*

**N.B Remember to always wear gloves when dealing with proteins to avoid contaminations!**

1. Following electrophoresis, remove the glass plate and transfer the gel into Transfer buffer.
2. Equilibrate the gel between 10-30min at room temperature on a shaker according to specific protocols.
3. Cut the membrane to the same size of the gel and equilibrate in Transfer buffer as well.

**NOTE: It is best to assemble the blotting sandwich in some transfer buffer to avoid the membrane to dry out and trapped bubble should be removed with a roller at each step during blotting sandwich assembly**

1. Each blot sandwich should be set up according to the following order:

- Cassette clamp -ve (black) side placed in a tray or other suitable container
- Pre-soaked fibre pad. Note two can be used with thin gels.
- Two pieces of thick filter paper, about 2 – 3 mm thick, pre-soaked in buffer.
- Gel.
- Transfer membrane. Usually this requires pre-soaking but consult the manufacturer's instructions for the type of membrane you are using. This should be smoothed so that no air bubbles have been trapped.
- Two pieces of thick filter paper, about 2 – 3 mm thick, pre-soaked in buffer.
- Pre-soaked fibre pad. Note two can be used with thin gels.
- Cassette clamp +ve (red).

2. Close the cassettes hinge carefully to not disturb the sandwich.
3. Fill the tank with buffer solution up to the maximum fill line indicated on the side of each unit. See recommended buffer solutions section.

**NOTE: Improved transfer can usually be obtained by using chilled buffer.**

| Buffer Volume | SB10 |
|---|---|
| One Cassette | 1380ml |
| Two Cassettes | 1290ml |
| Three Cassettes | 1200ml |
| Four Cassettes | 1110ml |

Each Cooling pack will take the place of ~ 100ml of buffer

## Blot Running Conditions

1. Insert the cassettes into the slots in the VS10BI blotting module with the black side of each adjacent to the negative electrode. It is a good idea to note the orientation and order the blot sandwiches were loaded in. This can be done by noting which samples were loaded adjacent to each electrode and by marking the membrane with a pencil on one corner.
2. Use of a magnetic stirring bar and plate is recommended to mix the buffer to give consistency of transfer. A 4mm diameter stirring bar should be placed underneath the module, in the centre of the tank. The Cooling pack provided, pre-frozen, can be inserted at the side or front of the tank for extended blots. Additional cooling packs can be purchased as accessories to further aid cooling.
3. Insert the blotting module in the tank making sure the polarity is correct, fit the lid and connect to a power supply.
4. Consult Suggested Running Condition for recommended power supply voltage settings and blot times. Please note voltages and current will

vary according to the number of cassettes, type and temperature of buffer and thickness and percentage of gel. This will also affect quality of transfer so time course of transfer should be performed for your particular samples and conditions.

5. When the blot time is completed, turn the power supply off.
6. Remove the cassettes from the main tank. Buffer can be re-used but this may affect run quality if continued.
7. Lift the hinge of each cassette and gently pry apart the blot sandwich and remove the membrane from the gel.
8. The membrane is now ready to be probed.

*Suggested Running Conditions (please note that these are just guideline)*

| **Duration of Blot** | **SB10** |
|---|---|
| One Hours | 100V – 125V<br>~ 250mA - 400mA |
| Three Hours | 50V<br>~ 200mA |
| Overnight ~16 hr | 10 V<br>30–40 mA |

N.B. If the voltage is held constant throughout a transfer, the current in most transfer systems increases as the resistance drops due to heating. Therefore, the overall power increases during transfer, and more heating occurs. Despite the increased risk of heating, a constant voltage ensures that field strength remains constant, providing the most efficient transfer possible for tank blotting methods. Use of the cooling elements available with the various tank blotting systems should prevent problems with heating.

# Solutions

**Stock 30% Acrylamide Gel Solution:-**

30.0 g acrylamide

0.8 g methylene bisacrylamide

Distilled Water to 100ml

**Stock 4 X Resolving Gel Tris (1.5 M Tris HCl pH8.8, 0.4 % SDS)**

To 110ml Distilled Water add 36.4 g of Tris base

Add 8ml of 10 % SDS

Adjust pH to 8.8 with 1N HCl

Adjust the final volume to 200ml with Distilled Water.

**Stock 4 X Stacking Tris (0.5 M Tris HCL pH6.8, 0.4 % SDS)**

To 110ml Distilled Water add 12.12 g of Tris base

Add 8ml of 10 % SDS

Adjust pH to 6.8 with 1N HCl

Add Distilled Water to a final volume of 200ml

**Stock 4 X Tris-glycine tank buffer - SDS**

36 g Tris base

172.8 g glycine

Distilled Water to 3 L

**1 x Tris-glycine tank buffer - SDS**

750ml of 4 X Tris-glycine reservoir buffer - SDS

30ml of 10 % SDS

Distilled Water to 3L

**10 % AP (ammonium persulphate solution)**

0.1 g ammonium persulphate

1ml Distilled Water

**TEMED ready solution**

**Stock 4 X Sample Buffer**

4ml glycerol

2ml 2-mercaptoethanol

1.2 g SDS

5ml 4 X Stacking Tris

0.03 g Bromophenol blue

Aliquot into 1.5ml microcentrifuge tubes. Store at -20°C.

## Towbin Buffer

25mM Tris,

192mM glycine,

20% methanol pH8.3,

## Towbin Buffer SDS

25mM Tris

192mM glycine

20% methanol pH8.3

0.05-0.1% (w/v) SDS

## Bjerrum and Schafer-Nielsen Buffer

48mM Tris

39mM glycine

20% methanol pH9.2

## Dunn Buffer

10mM $NaHCO_3$

3mM $NaCO_3$

20% methanol pH9.9

N.B. Do not adjust the pH when making these buffers as this will cause blot over-heating.

The pH will vary according to the freshness of the reagents used.

# References

1. Sambrook, Fritsch, and Maniatis, **Molecular Cloning A Laboratory Manual,** Second Edition,
Cold Spring Harbor Laboratory Press, 1989.

2. **Current Protocols in Molecular Biology,** Greene Publishing Associates and Wiley-Interscience,1989.

# Troubleshooting

| **SDS-PAGE electrophoresis** | | |
|---|---|---|
| Problem: Sample Preparation | Cause | Solution |
| Laemmil sample buffer turns yellow | Sample buffer too acidic | Add Tris base until buffer turns blue again. |
| Sample very viscous | High DNA or carbohydrate content | Fragment DNA with ultrasonic waves during cell lysis and protein solubilization.<br>Add endonucleases (for each benzonases).<br>Precipitate protein with TCA/acetone to diminish carbohydrate content. |
| **Problem: Gel casting and sample loading** | **Cause** | **Solution** |
| Poor well formation | Incorrect catalyst used<br>Monomer solution not degassed (oxygen inhibits polymerization) | Prepare Fresh catalyst solution.<br>Increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED.<br>Degas monomer solution immediately prior to casting stacking gel. |
| Webbing; excess acrylamide behind the comb | Incorrect catalyst concentration | Prepare fresh catalyst solution.<br>Increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED. |
| Gel does not polymerize | Too little or too much APS or TEMED<br>Failure to degas<br>Temperature too low<br><br>Poor quality acrylamide or bis<br>Old APS | Use 0.0.05% APS and 0.05% TEMED.<br>Degas monomer solutions 10-15min.<br>Cast at room temperature, warming glass plates if necessary.<br>Use electrophoreses-grade reagents<br>Prepare fresh APS. |
| Swirls in the gel | Excess catalysts; polymerization time < 10min<br>Gel inhibition; polymerization time >2hr | Reduce APS and TEMED by 25% each.<br>Increase APS and TEMED by 50%; degas. |
| Gel feels soft | Low %T<br>Poor quality acrylamide or bis<br>Too little cross-linker | Use different %T.<br>Use electrophoresis- grade reagents.<br>Use correct %C. |
| Gel turns white | Bis concentration too high | Check solutions or weights. |
| Gel brittle | Cross-linker too high | Use correct % cross-linker |
| Sample floats out of the well | Sample is not dense enough<br><br>Pipetting, loading error | Induce 10% glycerol in sample to make it denser than surrounding buffer.<br>Slowly pipet sample into well. Do not squirt sample quickly into well as it may bounce off bottom or sides and flow into next well. Do not pipet tip from well before last of sample has left the tip. |

| Problem: Electrophoresis | Cause | Solution |
|---|---|---|
| Current zero or less than expected and samples do not migrate into gel | Tape at the bottom of precast gel cassette not removed<br>Insufficient buffer in inner buffer chamber<br>Insufficient buffer in outer buffer chamber<br>Electrical disconnection | Remove tape.<br>Fill buffer chamber with running buffer.<br>Fill inner and outer buffer chambers to ensure wells are completely covered.<br>Check electrodes and connections. |
| Gels run faster than expected | Running buffer too concentrated and gel temperature too high; incorrect running buffer concentration or type used<br>Running or reservoir buffer too dilute<br>Voltage too high | Check buffer composition and type.<br>Check buffer protocol and concentrate if necessary.<br>Decrease voltage by 25-50%. |
| Gels run slower than expected | Incorrect running buffer composition or type<br>Excessive salt in sample | Check buffer composition and type.<br>Desalt sample. |
| **Problem: Total Protein Staining** | **Cause** | **Solution** |
| Bands not visible | No protein in gel<br>Imaging system malfunctioning<br>Incorrect imaging parameters were used | Stain with another method to confirm there is protein.<br>Check instrument manual for troubleshooting or contact imaging instrument manufacturer.<br>Check Instrument manual. |
| Poor staining sensitivity | Dirty staining trays<br>Insufficient stain volume<br>Insufficient staining time<br>Reuse of staining solution | Clean staining trays and other equipment with laboratory glassware cleaner.<br>Follow recommendations for stain volume (appropriate to gel size).<br>Increase staining time.<br>Repeat staining protocol with fresh staining solution. |
| High or uneven background staining | Staining trays or equipment dirty<br>Too much time in staining solution<br>Reagent impurities | Clean staining trays and other equipment with laboratory glassware cleaner.<br>Restrict duration of incubation in staining solutions as recommended in protocol.<br>Wash gel in water or retrospective destaining solution for >30min.<br>Use high-purity water and reagents for staining. |
| Speckles or blotches in gel image | Particulate material from reagents, staining tray, dust or gloves | Clean staining trays thoroughly.<br>Decrease time that gels and staining solution are exposed to open air.<br>Use dust-free gloves and handle gels only by edges. |
| Uneven staining | Insufficient shaking during staining | Agitate gel during staining. |
| Gel shrinkage | Gel dehydrated | Transfer gel to water for rehydration. |
| **Problem: Evaluation of Separation** | **Cause** | **Solution** |
| Diffuse or broad bands | Poor quality acrylamide or bis-acrylamide incomplete polymerization<br>Old SDS or sample buffer | Use electrophoresis-grade reagents.<br>Check polymerization conditions.<br>Prepare fresh solutions. |

| | | |
|---|---|---|
| | Gel temperature too high | Use external cooling during run or run out a lower voltage. |
| Bands 'smile' across gel, band pattern curves upward at both sides of gel | Excess heating of gel; centre of gel runs hotter than either end<br><br>Power conditions excessive<br><br>Insufficient buffer | Check buffer composition; buffer not mixed well, or buffer in upper chamber too concentrated.<br>Prepare new buffer, ensuring thoroughly mixing, especially when diluting 5x or 10x stock.<br>Do not exceed recommended running conditions. Decrease power setting from 200V to 150V or fill lower chamber to within 1cm of top of short plate.<br>Fill inner and outer buffer chambers to ensure that wells are completely covered. |
| Smiling or frowning bands with gel lane | Overloaded proteins<br>Sample preparation/ buffer issues<br><br>Incorrect running conditions | Load less protein.<br>Minimize salts, detergents and solvents in sample preparation and sample buffers.<br>Use correct voltage. |
| Skewed or distorted bands, lateral band spreading | Excess salt in samples<br><br>Ionic strength of sample lower than that of gel<br>Insufficient sample buffer or wrong formulation<br>Diffusion prior to turning on current<br><br>Diffusion during migration through stacking gel<br>Uneven gel interface | Remove salts, from sample by dialysis or desalting column prior to sample preparation.<br>Use same buffer in samples as in gel.<br><br>Check buffer composition and dilution instructions.<br>Minimize time between sample application and power start-up.<br>Increase %T of stacking gel to 4.5% or 5%T.<br>Increase current by 25% during stacking.<br>Decrease polymerization rate.<br>Overlay gels carefully.<br>Rinse wells after removing comb to remove residual acrylamide. |
| Vertical streaking | Overloaded samples<br><br>Sample precipitation | Dilute sample.<br>Selectively remove predominant protein in sample (fractionate).<br>Reduce voltage by 25% to minimize streaking.<br>Centrifuge samples to remove particulate prior to sample loading.<br>Dilute sample in sample buffer. |
| Fuzzy or spurious artefactual bands | Concentration of reducing agent too low | Use 5% BME or 1% DTT. |
| Protein bands do not migrate down as expected | Bands of interest may be neutral or positively charged in buffer used; pH of bands must be -2pH units more negative than pH of running buffer | Use SDS-PAGE or a different buffer system in native PAGE or IEF. |

| **Blotting** | |
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| **Poor protein transfer** | Transfer apparatus assembled incorrectly and proteins moving in the wrong direction.<br>• Gel/membrane sandwich may be assembled in the wrong order, or cassette inserted in wrong orientation. Check polarity.<br>• Air pockets not removed while assembling the blotting |

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| | sandwich |
| | Western detection system not working or not sensitive enough<br>• Include proper positive or negative control antigen. Consult kit manual.<br>• Use protein markers with coloured reference bands during PAGE.<br>• Stain gel with Coomassie, or stain membrane with Ponceau S. |
| | Transfer time too short – increase transfer time |
| | Power setting too low<br>• Increase Voltage<br>• Buffer may be prepared improperly – prepare new buffer and increase voltage. |
| | Charge-to-mass ratio incorrect for native transfers.<br>• Proteins close to isoelectric point (pI). Change buffer pH so that it is at least 2 pH unit higher or lower than pI of protein of interest. |
| | Defective or inappropriate power supply used.<br>• Check fuse of power supply. Ensure max. current output of power supply is at least 2000mA. |
| | Excessive methanol restricting transfer.<br>• Reduce methanol concentration to maximize protein transfer from gel, but without reducing concentration to the extent that it prevents binding to nitrocellulose. Alternatively reduce methanol concentration and switch to PVDF. |
| **Protein precipitating in gel** | • Use SDS in transfer buffer (SDS can increase transfer efficiency, but it can also reduce nitrocellulose binding affinity and affect protein-antibody reactivity).<br>• Remove alcohol from transfer buffer. |
| **Swirls or missing bands; diffuse transfers** | Poor gel-membrane contact. Air bubbles or excess buffer remain between membrane and gel.<br>• Carefully remove air bubbles between gel and membrane using a rolling pin<br>• Use more, or thicker, filter paper in gel-membrane sandwich<br>• Replace the fibre pads, as they degrade and remain permanently compressed over time. |
| | Problem with gel electrophoresis.<br>• Poor gel polymerization, inappropriate running conditions, buffer contamination, excessive sample application all contribute to poor quality gels and transfers. |
| **Gel cassette pattern transferred to blot** | Contaminated fibre pads<br>• Replace fibre pads or clean thoroughly.<br>Contaminated transfer buffer<br>• Replace buffer solutions. |
| **Poor binding to membrane** | Excessive methanol restricting transfer. |

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| **- nitrocellulose** | • Ensure methanol concentration does not exceed 20% (v/v). |
| | Proteins may be transferring through nitrocellulose.<br>• Use PVDF or smaller pore size (0.2µm) nitrocellulose.<br>• Overlay an extra piece of nitrocellulose over membrane to determine if proteins are migrating through the membrane directly in contact with the gel. |
| | Proteins <15kDa have reduced binding to 0.45µm nitrocellulose or may be washed from membrane during assays.<br>• Use PVDF or nylon membrane, which have higher binding capacities.<br>• Use Tween-20 detergent in the wash and antibody incubation steps. Reduce or eliminate the more stringent washing steps. |
| | SDS in transfer buffer reducing binding efficiency<br>• Reduce or eliminate SDS concentration |
| | Membrane incompletely wet<br>• White spots indicate dry areas where protein will not bind.<br>• If soaking does not occur immediately following immersion in transfer buffer, heat distilled water to just below boiling point and soak membrane until entirely wet. |
| **Poor binding to membrane PVDF** | Membrane is not completely wet<br>• Because of hydrophobicity of PVDF, the membrane must be soaked entirely in methanol before equilibration in aqueous buffer |
| | Proteins might be transferring through the membrane<br>• Decrease voltage if transferring under high intensity conditions<br>• Overlay an extra piece of PVDF over membrane to determine if proteins are migrating through the membrane directly in contact with the gel. |
| | Membrane might have dried during handling<br>• Fully wet membranes have a grey translucent appearance. White spots will form on the surface if the membrane has been allowed to dry. As proteins will not bind to dry spots, re-soak the membrane in methanol and re-equilibrate in transfer buffer |
| | SDS in transfer buffer reducing binding efficiency<br>• Reduce or eliminate SDS concentration |
| **Power is too high** | Always check current at the start of the run, for the current might be too high for a given voltage setting. Improper buffer preparation can also result in high conductivity and excessive power generation. The current setting should not be allowed to exceed 2000mA. |
| **Immune-specific detection** | Overall high background<br>• Reduce antibody / protein sample concentration |

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| | Too low background<br>• Increase antibody concentration / protein sample concentration<br>Consult manual included with antibody detection kit |
| **Total protein detection** | Consult stain or detection kit manual. |

# Warranty

The Cleaver Scientific Ltd. (CSL) omniPAGE Horizontal Electrophoresis units have a warranty against manufacturing and material faults of thirty-six months from date of customer receipt.

If any defects occur during this warranty period, CSL will repair or replace the defective parts free of charge.

This warranty does not cover defects occurring by accident or misuse or defects caused by improper operation.

Units where repair or modification has been performed by anyone other than CSL or an appointed distributor or representative are no longer under warranty from the time the unit was modified.

Units which have accessories or repaired parts not supplied by CSL or its associated distributors have invalidated warranty.

CSL cannot repair or replace free of charge units where improper solutions or chemicals have been used. For a list of these please see the Care and Maintenance subsection.

If a problem does occur, then please contact your supplier or Cleaver Scientific Ltd:

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