



## **Instruction Manual**

### **Verti-gel mini**

#### **Mini-Vertical Electrophoresis Systems**

FB79400 Verti-gel Complete Mini PAGE system

FB79401 Verti-gel Precast Mini system

FB79402 Verti-gel Modular PAGE & Electroblotting system

#### **2-D Electrophoresis Module**

FB79407 Mini Tube Gel internal module

#### **Electroblotting Module**

FB79408 Blotting internal module

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## **SAFETY PRECAUTION**



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 STATUTORY CE SAFETY DIRECTIVES:  
73/23/EEC: LOW VOLTAGE DIRECTIVE: IEC 1010-1:1990 plus AMENDMENT 1:1992  
EN 61010-1:1993/BS EN 61010-1:1993

## PACKING LISTS:

### Mini vertical gel systems FB79400/FB79401

Units include tank, lid, internal module and electrodes and include the following accessories:-

	<b>Glass Plates</b>	<b>Combs</b>	<b>Casting base</b>	<b>Cooling Pack</b>	<b>Cables</b>
<b>FB79400</b>	FB79425 (VS10NG) – Notched, Pk/2 FB79429 (VS10PGS1) – Plain with bonded 1mm spacers, Pk/2 FB79437 (VS10-DP) – Dummy Plate	2 of FB79465 (VS10-12-1) 1mm thick, 12 sample	FB79417 (VS10DCAST) FB79418 (VS10DCASTM) – Mat	FB79424 (VS10ICB)	1 Red, 1 Black
<b>FB79401</b>	FB79437 (VS10-DP) – Dummy Plate			FB79424 (VS10ICB)	1 Red, 1 Black

**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.**

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

## PACKING LISTS:

### Modular Blotting system FB79402

Units include tank, lid, internal module and electrodes and include the following accessories:-

	<b>Glass Plates</b>	<b>Combs</b>	<b>Casting base</b>	<b>Cooling Pack</b>	<b>Cables</b>
<b>FB79402</b>	FB79425 (VS10NG) – Notched, Pk/2 FB79429 (VS10PGS1) – Plain with bonded 1mm spacers, Pk/2 FB79437 (VS10-DP) – Dummy Plate	2 of FB79465 (VS10-12-1) 1mm thick, 12 sample	FB79417 (VS10DCAST) - FB79418 (VS10DCASTM) – Mat	FB79424 (VS10ICB)	1 Red, 1 Black
	<b>Blotting Module</b>	<b>Cassettes</b>	<b>Fibre pads</b>		
	<b>FB79408</b>	4 of FB79422 (SB10C)	1 of FB79423 (SB10F), Pk/8		

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.

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

## PACKING LISTS:

### Mini-tube gel module FB79407

Includes internal 2-D capillary gel module, glass capillaries and Blanking plugs as follows:-

<b>Tube Gel Module</b>	<b>Capillary Tubes</b>	<b>Blanking Plugs</b>
<b>FB79407</b>	FB79420 (MCT10)	FB79421 (MCBP)

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.

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

### Electroblotting module FB79408

Includes internal Electroblotting module cassettes and fibre pads as follows:-

<b>Electroblotting Module</b>	<b>Blotting Cassettes</b>	<b>Fibre pads</b>
<b>FB79408</b>	4 of FB79422 (SB10C)	FB79423 (SB10F) Pk/8

## **Care and Maintenance:-**

### **Cleaning Verti-gel Units**

Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60° 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 (H<sub>2</sub>O<sub>2</sub>) for 10 minutes.

Rinse with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water.

**Caution:** DEPC is a suspected carcinogen. Always take the necessary precautions when using.



## 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 Verti-gel Tanks:-

### Instructions for fitting Electrode Cables.

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 to 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.

**The unit is now ready to be used.**

## **Vertical Gel Casting Using the Verti-gel Gel Casting System:-**

**See page 12 for diagrams detailing the Mini vertical gel casting procedure.**

1. 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. **All glass plates, modules and casting base accessories must be completely dry during set – up. Wet components are more likely to miss-align and cause leaks.**
2. Assemble the glass plates so that the bottom of the glass plates and the spacers are perfectly aligned. 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: The glass plates with bonded spacers have an arrow in the top of the spacers which are slightly longer than the glass plate to indicate the top.**
3. The Slab Gel Insert contains pressure bars which impart even pressure onto the glass plates and allow even pressure transfer onto the sealing edge of the glass plate, ensuring complete sealing. Ensure that the pressure bars are adequately open for the thickness of spacer used. The bar can be opened by sliding the clamps. When using a triple glass plate sandwich, the pressure bars will need to be in the completely open position.
4. Position the Slab Gel Insert on a flat surface. **Do not at this stage insert the Slab Gel Insert into the casting base.**

5. Insert the glass plates into the Slab Gel Insert between the pressure bar and the blue gasket and fully tighten the pressure bar by simply sliding 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. **At this stage, check that the bottom edges of the spacers and glass plates are perfectly aligned.**
6. 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.
7. With the cam pin handles facing directly downwards, turn the cam pins fully through 180<sup>0</sup> or until the insert has tightened onto the silicone mat.

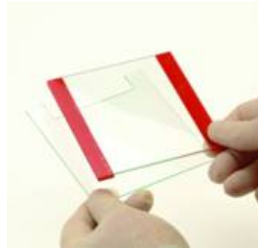
**It is best to turn the cams in opposite directions to each other. Do not overturn as this will cause the glass plates to push upwards and the assembly will be more likely to leak. The unit is now ready for gel preparation and pouring**

**Always reverse the silicone mat after casting to avoid indentations from persistent use. 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 unit 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.**

## VERTICAL GEL CASTING.

1) Put together bonded spacer plain glass plate with notched plate



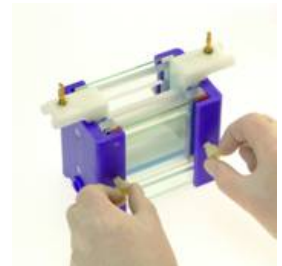
2) Insert assembled cassette between pressure bar and blue gasket with notched plate facing inwards



3) Slide clamps outwards to firmly secure the cassette in the module between pressure bar and gasket



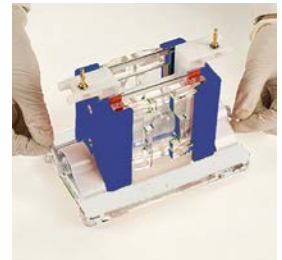
3) Alternatively if using the optional screws tighten to secure the gel



4) Insert assembled module and cassette into the casting base



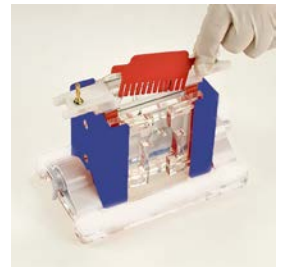
5) Push the cams in to the holes. Turn cams about 90° to securely seat the unit on the silicone mat. Do not overtighten.



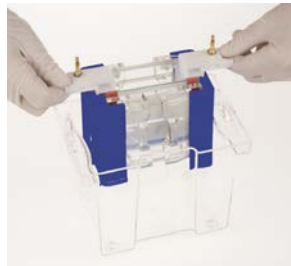
6) Pour resolving gel into unit and allow to set.



7) Add stacking gel and insert comb, allow to set



8) Remove gel module from casting base. Insert module into outer tank with correct alignment. Add required buffers.



9) Put on gel tank lid and connect to power supply at required voltage.



## Gel Preparation:-

1. It is always advisable to work using stock solutions which allow added convenience and save time when it comes to gel pouring. Pages 21 and 22 list stock solutions for SDS PAGE gels which should be pre-made beforehand. For native gel formulae and running conditions, please consult a laboratory manual. The protocol below is given for use of the standard stock solutions advised. This should be adjusted if you are using different stock solutions or gel formulas.

2. Table 1 below shows the total volume of gel solution required. In subsequent tables, amounts of gel and solutions are given for two 1mm thick gels so adjustments are needed when running single, or more than two gels and for 0.75, 1.5 or 2mm thick spacers.

**Table 1.**

<b>Verti-gel Mini – FB79400</b>	
	Total Gel volume for a 1mm thick gel.
For different thicknesses of gel, multiple the below amounts by the spacer thickness.	
Single – one gel, one dummy plate	7.5ml
Double – two gels	15ml
Using a Triple Plate sandwich – four gels	30ml

## Gel Selection:-

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 for different sizes of protein.

See Table 2 below which details which percentage of gel to use to separate the sizes of proteins indicated.

**Table 2.**

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

3. 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.

**Table 3: Preparation of the separating gel solution for two 10 x 10cm FB79400 gels using 1 mm spacers.**

<b>Solution</b>	<b>5 %</b>	<b>7.5%</b>	<b>10 %</b>	<b>12%</b>	<b>15 %</b>	<b>17.5%</b>
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
10 % Ammonium Persulphate	150µl	150µl	150µl	150µl	150µl	150µl

## **Gel Pouring:-**

### **For gels with stacking layers:-**

4. Insert the comb into the glass plates and mark a point on the glass plates 1cm below where the comb teeth finish. This indicates the fill level for the resolving gel.
5. Add 15µl of TEMED to the resolving gel solution for FB79400 sized gels and mix well but avoid generating air bubbles.
6. Fill the glass plates again avoiding generating any air bubbles. Filling must be performed quickly before the TEMED causes the gel to become too viscous.
7. 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.
8. Let the resolving gel polymerize. Usually this takes around 15 minutes but this can vary due to the freshness of the reagents used. If polymerization is taking a lot longer than this, use fresher stock solutions or add more APS and TEMED.
9. Prepare the stacking gel using Table 4 below as a guide. Again stock solutions are given on pages 17 and 18.

**Table 4. Stacking gel preparation**

<b>Solution</b>	<b>FB79400</b>
Distilled Water	4.2ml
30 % Stock Acrylamide Solution	0.65ml
4 X Stacking Gel Tris Solution	1.6ml
10 % Ammonium Persulphate	67 $\mu$ l

10. Carefully mix the stacking gel solution, avoiding generating air bubbles.
11. Pour off the overlay liquid and rinse the gel with distilled water.
12. Add 6.7 $\mu$ l of TEMED to the stacking gel solution for FB79400 gels. Use a Pasteur pipette to fill the glass plates up to the top with stacking gel solution.
13. 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.
14. Allow the stacking gel to polymerize for 30 minutes.

**For gels without stacking layers:-**

4. Add 15 $\mu$ l of TEMED to the resolving gel solution for FB79400 sized gels and mix well but avoid generating air bubbles.
5. Fill the glass plates again avoiding generating any air bubbles. Filling must be performed quickly before the TEMED causes the gel to become too viscous.
6. 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.

7. Let the gel polymerize. Usually this takes around 15 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.

**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 (See Comb specifications page 31).
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 manufacturers 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.

## Loading the samples:

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.

**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 1 x reservoir buffer. See page 21 for recommended running buffer solution. Table 5. on page 19 shows the volume of buffer required.
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.

**Table 5.**

<b>Buffer Volume</b>	<b>FB79400/FB79401</b>
<p><b>Minimum</b> – Inner tank is filled to above the wells. Outer Tank is filled to just flood the bottom of the glass plates. Cooling potential is at a minimum which may affect resolution.</p>	250ml
<p><b>Maximum</b> – Inner tank is filled to above the wells. Outer Tank is filled to the maximum fill line. Cooling is high offering good resolution of samples.</p>	1200ml
<p><b>Using the cooling packs</b> – Inner tank is filled to above the wells. Cooling packs are inserted behind the gels. Outer Tank is filled to the maximum fill line. Cooling is at a maximum.</p>	1000ml

## Gel Running:

1. Fit the lid and connect to a power supply.
2. See Table 6 for details of recommended power supply voltage settings.
3. Turn the power supply off when the loading dye reaches the bottom of the gel, sooner if your proteins are below 4KD in size.
4. 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.
5. Unscrew the glass plates and gently pry apart the glass plates. 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.
6. 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.

**Table 6.**

<b>Recommended Voltages and Resultant Current for 1mm thick, 12% gels.</b>	<b>FB79400/FB79401</b>
One gel	90-225V 20-45mA
Two gels	90-225V 40-90mA
Three gels	90-225V 60-135mA
Four gels	90-225V 80-180mA

## **Stock Solutions for SDS PAGE gels:-**

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

30.0 g acrylamide

0.8 g methylene bisacrylamide

Distilled Water to 100ml

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

36 g Tris base

172.8 g glycine

Distilled Water to 3 L

### **1X 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****Stock 4X Sample Buffer**

4ml glycerol

2ml 2-mercaptoethanol

1.2 g SDS

5ml 4X Stacking Tris

0.03 g Bromophenol blue

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

**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.

## **1<sup>st</sup> Dimension Electrophoresis using the Verti-gel Tube Gel Module (FB79407)**

### **Capillary Tube Gel Pouring:-**

There are two methods that can be used for tube gel casting. Method 1 details casting by injection, method 2 details casting by capillary action.

#### **Method 1:- Filling By Injection**

1. Place the appropriate number of capillary tubes into the Tube Gel Running module, inserting these carefully from the top.

2. Seal the bottom ends of the tubes using NescoFilm.

3. Prepare the following solution. This will be enough to pour twenty 80mm Capillary Tubes. For Native IEF Gels, do not use Urea and NP-40 and use 18ml of distilled water instead of 16ml;  
16ml Distilled Water (18ml for Native Gels)

2.4ml Glycerol

0.9ml Commercially available 40% ampholyte solution

3.8ml Acrylamide/Bis solution

15µl TEMED

16.2g Urea (omit for Native Gels)

0.6ml NP40 (omit for Native Gels)

This solution should be de-gassed prior to pouring.

When ready to pour, add 120µl of 10% w/v ammonium persulphate solution.

4. Using a Hamilton, or similar, syringe, insert the needle into the tube and carefully inject the solution so that the tubes fill from the bottom. Keep filling to 1cm of the length of the tubes. The tubes can be gently tapped to get rid of air bubbles.

5. Fill the remaining 1cm gap with water saturated isobutanol.

6. Leave to fully polymerise, which will normally take 1 – 2 hours.

7. After polymerisation, remove the water-saturated isobutanol. Tube gels can be used immediately or stored wrapped in a damp paper towel and Nescofilm at 4°C. The Nescofilm at the bottom of the tubes must be removed prior to electrophoresis.

### **Method 2:- Filling By Capillary Action**

1. Place the appropriate amount of capillary tubes in a suitable outer receptacle such as a 15ml falcon tube.
2. The amount of acrylamide required depends on the size of the outer receptacle used. The larger the outer receptacle used, the more acrylamide wastage so the following advised volumes may need to be increased.

Prepare the following solution. This will be enough to pour twenty 80mm Capillary Tubes. For Native IEF Gels, do not use Urea and NP-40 and use 18ml of distilled water instead of 16ml;

32ml Distilled Water (18ml for Native Gels)

4.8ml Glycerol

1.8ml Commercially available 40% ampholyte solution

7.6ml Acrylamide/Bis solution

30µl TEMED

32.4g Urea (omit for Native Gels)

1.2ml NP40 (omit for Native Gels)

This solution should be de-gassed prior to pouring.

When ready to pour, add 240µl of 10% w/v ammonium persulphate solution.

3. Fill the falcon tube with 70% of the acrylamide solution. The capillary tubes will fill by capillary action.
4. Allow the tubes to equilibrate for a few moments.
5. Check the height of the acrylamide in the tubes. If the tubes are full so that there is less than a 1cm non-filled space at the top, remove some of the acrylamide solution from the beaker until the height is 1 cm from the top. If there is a greater than 1cm space at the top, add more acrylamide solution, so that the solution rises in the tubes until there is a 1cm space at the top.

6. When the solution has reached to within 1cm of the top of the tube, stop adding the acrylamide solution.
7. Fill the remaining 1cm gap with water saturated isobutanol.
8. Leave to fully polymerise, which will normally take 1 – 2 hours.
9. After polymerisation, remove the water-saturated isobutanol. Tube gels can be used immediately or stored wrapped in a damp paper towel and Clingfilm at 4°C.
10. The tubes may contain a residual of acrylamide on the outside and may need cleaning with distilled water before insertion into the tube gel insert.

## **1st Dimension (IEF) Phase Tube Gel Running**

Buffer and run conditions will vary according to the type of ampholyte used. The following conditions are given as an example. Ampholytes require different buffer solutions dependent on the formulation. Please consult the manufacturer's instructions.

1. Prepare ~ 500ml of 10mM H<sub>3</sub>PO<sub>4</sub> Anode Buffer and use this to fill the bottom chamber of the unit so that the bottoms of the capillary tubes are submerged. If less than 10 capillary tubes are to be run, block up the unused tube slots in the internal running module with the blanking plugs provided. For high resolution separations, we recommend filling the lower chamber completely with buffer and using a pre-frozen cooling pack(s).
2. Place the Internal running Module into the unit and fill the upper buffer reservoir with ~100 mls of 20mM NaOH Cathode Buffer so that the tops of the capillary tubes are submerged.
3. For the Prefocus, load the gels with 10µl of 1% ampholyte solution and run for 15 minutes at 200V, then for 30 minutes at 300 V and then finally 30 minutes at 400V. The Prefocus stage is recommended as it helps set up the pH gradient.
4. Load the tubes with the samples. These should be dissolved in 1% ampholyte with 20% glycerol.
5. Replace the safety lid firmly making sure that the electrical connectors form a good contact.
6. Connect the electrophoresis apparatus to the power pack and connect the power pack to the mains supply. Turn all settings to zero before turning on the mains supply.

7. Run at 400V for 3 hours and then 800V for 30 minutes. These conditions are for 8cm tubes.
8. At the end of the run, turn the power supply settings to zero, turn off the mains supply and disconnect the power leads.
9. Remove the Internal Module and remove the tubes from their slots. The gels can be extracted from the capillary tubes by: **a)** inserting a piece of wire with a small plug of cotton wool on the end and using this as a piston to push the gel out, **b)** inserting a Gilson tip into the end of the gel and gently squeezing the gel out with air or water. Whichever of these two methods is used, the gels should be handled with care as they are fragile.

## **2-D, Size Determination Phase**

1. To prepare the tube gel(s) for the 2-D, size-determining phase, equilibrate them by soaking for 30 minutes in the running buffer to be used for the 2-D phase.
2. Remove the gel(s) from the running buffer pre-soak, and place each lengthways onto the top of a pre-poured slab gel. The slab gel should be cast using a blank or 2-D comb. For details on the casting of slab gels see the previous pages in this manual.
3. Hold the tube gel in place by pouring over it a low % agarose gel containing the tracker dye.
4. Electrophoresis should be performed as for Slab Gels until the tracker dye has advanced the required distance down the gel.
5. The samples can be visualized using any of the standard staining methods or can be blotted.

## Protein Blotting using the Verti-gel Electroblotter module (FB79408)

**Setting up the blot sandwich:** The most commonly used buffer solutions are given on page 30.

1. Each blot sandwich should be set up as follows:-
  - a. Cassette clamp -ve (black) side placed in a tray or on other suitable surface.
  - b. Pre-soaked fibre pad. **Note two can be used with thin gels.**
  - c. Two pieces of thick filter paper, about 2 – 3 mm thick, pre-soaked in buffer.
  - d. Gel.
  - e. Transfer membrane. Usually this requires pre-soaking but consult the manufacturers instructions for the type of membrane you are using. This should be smoothed so that no air bubbles have been trapped.
  - f. Two pieces of thick filter paper, about 2 – 3 mm thick, pre-soaked in buffer.
  - g. Pre-soaked fibre pad. **Note two can be used with thin gels.**
  - h. Cassette clamp +ve (red) side slotted into the groove in the bottom of the black cassette.
2. Close the hinge carefully so as 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 page 30 for recommended buffer solutions. Improved transfer can usually be obtained by using chilled buffer.

**Table 7. Volume of buffer required for each unit (cassette number dependant).**

Buffer Volume	FB79402
One Cassette	1380ml
Two Cassettes	1290ml
Three Cassettes	1200ml
Four Cassettes	1110ml

Each Cooling pack will take the place or 100ml of buffer for FB79402.

## **Blot Run Conditions:**

1. Insert the cassettes into the slots in the 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.
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 module, fit the lid and connect to a power supply.
4. Consult Table 8, Page 29 for details on recommended power supply voltage settings and blot times. Please note voltages and current will vary according to the amount 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 prise apart the blot sandwich and remove the membrane from the gel.
8. The membrane is now ready to be probed.

**Table 8. Recommended voltages and average resultant current.**

<b>Duration of Blot</b>	<b>FB79402</b>
One Hours	100V 400mA
Three Hours	50V 200mA

## **References:-**

1. **Molecular Cloning A Laboratory Manual**, Sambrook, Fritsch, and Maniatis, Second Edition, Cold Spring Harbor Laboratory Press, 1989.
2. **Current Protocols in Molecular Biology**, Greene Publishing Associates and Wiley-Interscience, 1989.
3. **Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications**, Towbin, J., Staehelin, T., and Gordon, J. (1979). Proc. Natl., Acad. Sci. USA, 76, 4350-4354.
4. **Blotting Techniques Ch.1, 7.10, p. 85-97. In: Gel Electrophoresis of Proteins, A Practical Approach**, B.D.Hames and D.Rickwood, eds., IRL Press. (1990),

## **BUFFER SOLUTIONS:-**

### **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<sub>3</sub>  
3mM NaCO<sub>3</sub>  
20% methanol pH9.9

**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.**



**Combs:–**

**MC Denotes Multi Channel Pipette compatible.**

**FB97400, FB97401:–**

Fisher Code	Description	Sample Volume $\mu$ l per well
FB79452	Comb 1 Prep, 1 Marker, 0.75mm thick	500
FB79453	Comb 5 sample, 0.75mm thick	70
FB79454	Comb 8 sample MC, 0.75mm thick	40
FB79455	Comb 9 sample, 0.75mm thick	35
FB79456	Comb 10 sample, 0.75mm thick	30
FB79457	Comb 12 sample, 0.75mm thick	25
FB79458	Comb 16 sample MC, 0.75mm thick	20
FB79459	Comb 20 sample, 0.75mm thick	15
FB79460	Comb 1 Prep, 1 Marker, 1mm thick	650
FB79461	Comb 5 sample, 1mm thick	100
FB79462	Comb 8 sample MC, 1mm thick	60
FB79463	Comb 9 sample, 1mm thick	50
FB79464	Comb 10 sample, 1mm thick	40
FB79465	Comb 12 sample, 1mm thick	35
FB79466	Comb 16 sample MC, 1mm thick	25
FB79467	Comb 20 sample, 1mm thick	20
FB79468	Comb 1 Prep, 1 Marker, 1.5mm thick	1000
FB79469	Comb 5 sample, 1.5mm thick	140
FB79470	Comb 8 sample MC, 1.5mm thick	80
FB79471	Comb 9 sample, 1.5mm thick	70
FB79472	Comb 10 sample, 1.5mm thick	30
FB79473	Comb 12 sample, 1.5mm thick	50
FB79474	Comb 16 sample MC, 1.5mm thick	40
FB79475	Comb 20 sample, 1.5mm thick	30
FB79476	Comb 1 Prep, 1 Marker, 2mm thick	1300
FB79477	Comb 5 sample, 2mm thick	200
FB79478	Comb 8 sample MC, 2mm thick	120
FB79479	Comb 9 sample, 2mm thick	100
FB79480	Comb 10 sample, 2mm thick	80
FB79481	Comb 12 sample, 2mm thick	70
FB79482	Comb 16 sample MC, 2mm thick	50
FB79483	Comb 20 sample, 2mm thick	40

**Notes**



## Warranty

Fisher Scientific Verti-gel Vertical Electrophoresis units have a warranty against manufacturing and material faults of twelve months from date of customer receipt.

If any defects occur during this warranty period, Fisher Scientific 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 Fisher Scientific are no longer under warranty from the time the unit was modified.

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

Fisher Scientific 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 nearest Fisher Scientific supplier.