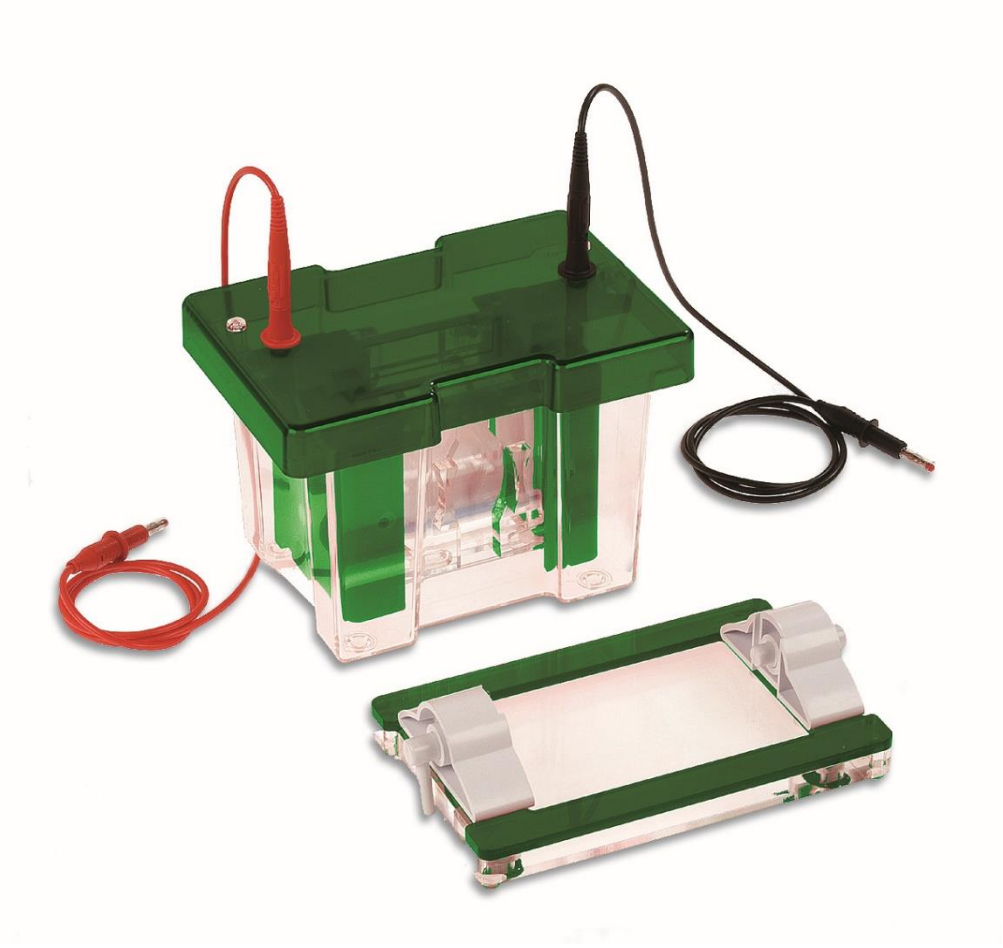
EVS1000 series

MANUAL

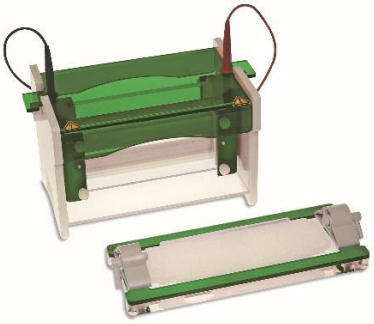
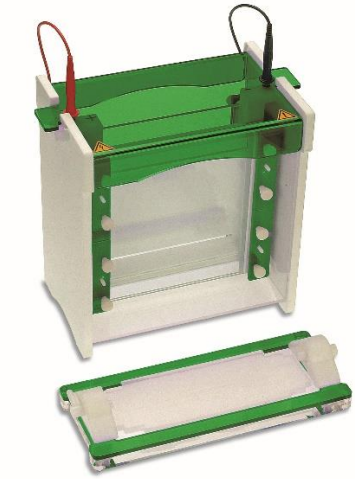
Rev. 44/2019

Gel Electrophoresis
Vertical units



Consort

Models

	Plate dimensions (cm)	Gel dimensions (cm)	Number of Gels	Sample Capacity	
EVS1100	10x10	7.5x8	1 to 4	80 (20 per gel)	
EVS1200	20x10	18x8	1 to 4	192 (48 per gel)	
EVS1300	20x20	16x17.5	1 to 4	192 (48 per gel)	

Contents

Safety Information

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.
- Always isolate electrophoresis units from their power supply before removing the safety cover. Isolate the power supply from the mains first then disconnect the leads.
- Do not exceed the maximum operating voltage or current.
- Do not operate the electrophoresis units in metal trays.
- Acrylamide is a volatile, cumulative neurotoxin and suspected carcinogen. Wear effective protective clothing and follow recommended handling and disposal procedures.
- Polymerised gels contain some unpolymerised monomer. Handle with gloves only. Following the replacement of a platinum electrode have the unit inspected and approved by your safety officer prior to use.
- Do not fill the unit with running buffer above the maximum fill lines.
- Do not move the unit when it is running.
- **Caution**: during electrophoresis very low quantities of various gases are produced at the electrodes. The type of gas produced depends on the composition of the buffer employed. To disperse these gases make sure that the apparatus is run in a well ventilated area.

General care and maintenance

- 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 inner module should be thoroughly rinsed with warm water or distilled water to prevent buildup 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 dish washing 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.
- **In case of Rnase Decontamination**
  Clean the units with a mild detergent as described above.
  Wash with 3 % hydrogen peroxide (H202) 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

Environmental conditions

- This apparatus is intended for indoor use only.
- This apparatus can be operated safely at an altitude up to 2000 m.
- The normal operating temperature range is 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.
- The 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 vertical gel tank

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.

Gel casting

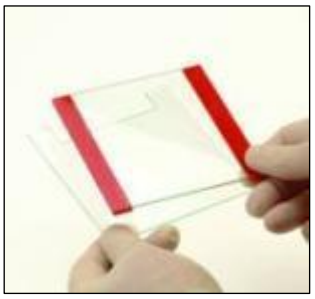
Cleaning the Glass Plates

- 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.

Glass cassette Assembly

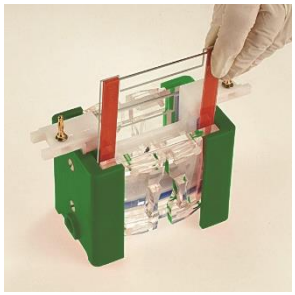
- Assemble the glass plates so that the bottom of the glass plates and the spacers are perfectly aligned.
- For triple plate sandwiches, the free spacers need to be perfectly aligned which is best performed using a small spacer or comb to push the spacers apart. Notched glass plates with bonded spacers 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.

Casting Stand Assembly

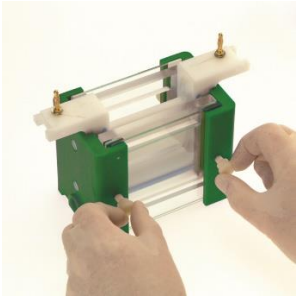
- Position the Slab Gel Insert on a flat surface. Do not at this stage insert the slab gel insert into the casting base.
- Insert the glass plates into the Slab Gel Insert between the pressure bar and the blue gasket.



- 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.

  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.

  Then fully tighten the pressure bar screws in the order top then bottom.



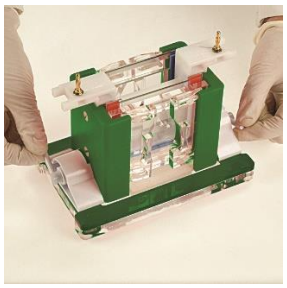
- Fully tighten the screw for the Mini vertical and the screws sequentially and in an even manner for the maxi vertical in the order middle two, top then bottom, making sure not to wobble the unit.

  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.

- 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 GRM may need to be pushed down very slightly to locate the cam pins.



- With the cam pin handles facing directly downwards, turn the cam pins fully through 180° or until the insert has tightened onto the silicone mat.

Notes:

- 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 EVS1100 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.

Gel preparation

1. It is always advisable to work with pre-made stock solutions which allow added convenience and save time when it comes to gel pouring. 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 below shows the volume of agarose solution required to make the desired gel (1 mm thick) for each unit size. Adjustments are needed for 0.75, 1.5 or 2 mm spacers.

Model	Gel size (cm)	Gel Volume (ml)
EVS1100	10x10 (one gel + dummy plate) 10x10 (two gels) 10x10 (four gels + triple plates)	7.5 15 30
EVS1200	20x10 (one gel + dummy plate) 20x10 (two gels) 20x10 (four gels + triple plates)	17.5 35 70
EVS1300	20x20 (one gel + dummy plate) 20x20 (two gels) 20x20 (four gels + triple plates)	35 70 140

Gel selection

1. 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. See table below which details and which percentage of gel to use to separate the sizes of proteins indicated.

Acrylamide percentage	**Separating resolution**
5%	60...220 kDa
7.5%	30...120 kDa
10%	20...75 kDa
12%	17...65 kDa
15%	15...45 kDa
17.5%	12...30 kDa

2. 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.

Preparation of the separating gel solution for 10x10 cm gels using 1 mm spacers.

Solution	**5%**	**7.5%**	**10%**	**12%**	**15%**	**17.5%**
distilled water	8.7 ml	7.5 ml	6.3 ml	5.25 ml	3.75 ml	2.5 ml
30% stock acrylamide	2.5 ml	3.75 ml	5 ml	6 ml	7 ml	8.75 ml
4x resolving Tris	3.75 ml	3.75 ml	3.75 ml	3.75 ml	3.75 ml	3.75 ml
10% ammonium persulphate	0.15 ml	0.15 ml	0.15 ml	0.15 ml	0.15 ml	0.15 ml

Preparation of the separating gel solution for 20x10 cm gels using 1 mm spacers.

Solution	**5%**	**7.5%**	**10%**	**12%**	**15%**	**17.5%**
distilled water	20.5 ml	17.6 ml	14.8 ml	12.3 ml	8.8 ml	5.8 ml
30% stock acrylamide	5.8 ml	8.8 ml	11.7 ml	14.1 ml	17.6 ml	20.5 ml
4x resolving Tris	8.8 ml	8.8 ml	8.8 ml	8.8 ml	8.8 ml	8.8 ml
10% ammonium persulphate	0.35 ml	0.35 ml	0.35 ml	0.35 ml	0.35 ml	0.35 ml

Preparation of the separating gel solution for 20x20 cm gels using 1 mm spacers.

Solution	**5%**	**7.5%**	**10%**	**12%**	**15%**	**17.5%**
distilled water	41 ml	35.3 ml	29.6 ml	24.7 ml	17.6 ml	11.7 ml
30% stock acrylamide	11.7 ml	17.6 ml	23.5 ml	28.2 ml	35.3 ml	41.1 ml
4x resolving Tris	17.6 ml	17.6 ml	17.6 ml	17.6 ml	17.6 ml	17.6 ml
10% ammonium persulphate	0.70 ml	0.70 ml	0.70 ml	0.70 ml	0.70 ml	0.70 ml

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 polymerize.
12. Once the gel is polymerized it is ready for the electrophoresis run.

Casting a gel without stacking layer

1. Prepare the resolving gel solution. Mix well and avoid generating air bubbles.
2. Pour the solution smoothly into the glass plates avoiding any air bubbles until the top of the notched glass plate is reached.
3. 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.
4. 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.
5. Once the gel is polymerized it is ready for the electrophoresis run.

Using Precast Gels

1. The mini vertical unit 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.

Preparation of denaturated 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 4x 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 12000 rpm. The protein samples are now ready to load.

Module 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.

   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 slower 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 further for recommended running buffer solution. Check the table her below for the required volume:

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	EVS1100	EVS1200	EV1300
Minimum – 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.	250 ml	500 ml	1200 ml
Maximum – 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.	1200 ml	2800 ml	5600 ml
Using the cooling packs – 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.	1000 ml	2300 ml	4600 ml

Gel Running

1. Fit the lid and connect to a power supply.
2. Consult the table for details on 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 with the Screw version. To open the sliding door version insert the key into the recess arch of the clamping door. Twist key applying pressure to both the clamping door and the side cheek. The door will now click open. Repeat this process until you have opened both the doors.
6. Remove the glass plates. Then using the key to 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.
7. 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.

Recommended Voltages and Resultant Current for 1mm thick, 12% gels.	**EVS1100 EVS1200**	**EVS1300**
One gel	90 - 225 V 20 - 45 mA	120 - 250 V 20 - 45 mA
Two gels	90 - 225 V 40 - 90 mA	120 - 250 V 40 - 90mA
Three gels	90 - 225 V 60 - 135 mA	120 - 250 V 60 - 135 mA
Four gels	90 - 225 V 80 - 180 mA	120 - 250 V 80 - 180 mA

Solutions for SDS PAGE gels

Stock 30% acrylamide gel solution

- 30.0 g acrylamide
- 0.8 g methylene bisacrylamide
- add distilled water to a final volume of 100 ml

Stock 4 x resolving gel Tris (1.5 M Tris.HCl pH 8.8, 0.4 % SDS)

- to 110 ml distilled water add 36.4 g of Tris base
- add 8 ml of 10 % SDS
- adjust pH to 8.8 with 1 M HCl
- add distilled water to a final volume of 200 ml

Stock 4 x stacking Tris (0.5 M Tris.HCl pH 6.8, 0.4 % SDS)

- to 110 ml distilled water add 12.12 g of Tris base
- add 8 ml of 10 % SDS
- adjust pH to 6.8 with 1 M HCl
- add distilled water to a fi nal volume of 200 ml

Stock 4 x Tris-glycine tank buffer - SDS

- 36 g Tris base
- 172.8 g glycine
- add distilled water to a final volume of 3000 ml

1 x Tris-glycine tank buffer - SDS

- 750 ml of 4 x Tris-glycine reservoir buffer - SDS
- 30 ml of 10 % SDS
- add distilled water to a final volume of 3000 ml

10 % AP (ammonium persulphate solution)

- 0.1 g ammonium persulphate
- ml distilled water

TEMED

Stock 4 x sample buffer

- 4 ml glycerol
- 2 ml 2-mercaptoethanol
- 1.2 g SDS
- 5 ml 4 x stacking Tris
- 0.03 g bromophenol blue
- aliquot into 1.5 ml microcentrifuge tubes.
- Store at -20°C

2-D electrophoresis

Capillary tube gel pouring

There are two methods which 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:
   - 16 ml distilled water (18 ml for native gels)
   - 2.4 ml glycerol
   - 0.9 ml 4-8 resolyte or other commercially available 40 % ampholyte solution
   - 3.8 ml acrylamide/bis solution
   - 15 µl TEMED
   - 16.2 g urea (omit for native gels)
   - 0.6 ml NP40 (omit for native gels)

   This will be enough to pour twenty 80 mm capillary tubes or ten 170 mm capillary tubes. This solution should be de-gassed prior to pouring. When ready to pour, add 120 µl 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 fi ll from the bottom. Keep filling to 1 cm of the length of the tubes. The tubes can be gently tapped to get rid of air bubbles.
5. Fill the remaining 1 cm 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 number of capillary tubes in a suitable outer receptacle such as a 15 ml 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.
3. Prepare the following solution:
   - 32 ml distilled water (18 ml for native gels)
   - 4.8 ml glycerol
   - 1.8 ml 4-8 resolyte or other commercially available 40 % ampholyte solution
   - 7.6 ml acrylamide/bis solution
   - 30 µl TEMED
   - 32.4 g urea (omit for native gels)
   - 1.2 ml NP40 (omit for native gels)

   This will be enough to pour twenty 80 mm capillary tubes or ten 170 mm capillary tubes. This solution should be de-gassed prior to pouring. When ready to pour, add 240 µl of 10 % w/v ammonium persulphate solution.

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 1 cm 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 1 cm space at the top, add more acrylamide solution, so that the solution rises in the tubes until there is a 1 cm space at the top.
6. When the solution has reached to within 1 cm of the top of the tube, stop adding the acrylamide solution.
7. Fill the remaining 1 cm 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 Nescofilm at 4°C. The Nescofilm at the bottom of the tubes must be removed prior to electrophoresis.
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 guidelines only and apply when 4-8 Resolyte is the ampholyte used. Other Ampholytes will require different buffer solutions. Please consult manufacturer's instructions.

1. Prepare ca. 500 ml of 10 mM H_3PO_4 Anode Buffer (1 litre for EVS1200, 2 litres for EVS1300) 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 ml of 20 mM NaOH cathode buffer (200 ml for E4200, 400 ml for E4300) so that the tops of the capillary tubes are submerged.
3. For the prefocus, load the gels with 10 µl of 1 % ampholyte solution and run for 15 minutes at 200 V, then for 30 minutes at 300 V and then finally 30 minutes at 400 V. 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 400 V for 3 hours and then 800 V for 30 minutes. These conditions are for 8 cm tubes. 17 cm tubes need to be run at 400 V for 18 hours and then 800 V for 1 hour.
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:
    - 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.
    - 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. Electrophorese as usual 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.

Electroblotting

Setting up the blot sandwich

1. Each blot sandwich should be set up as follows:
   - Cassette clamp [-ve] (black) side placed in a tray or other suitable surface.
   - 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) 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. Improved transfer can usually be obtained by using chilled buffer.

Buffer Volume	EVS1100	EVS1200	EV1300
One cassette	1380 ml	2800 ml	5600 ml
Two cassettes	1290 ml	2620 ml	5240 ml
Three cassettes	1200 ml	2440 ml	4880 ml
Four cassettes	1110 ml	2260 ml	4520 ml

Each cooling pack will take the place of 100 ml of buffer for EVS1100 and 500 ml of buffer for EVS1200 and EVS1300.

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 4 mm 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 below table for details on recommended power supply voltage settings and blot times. Please note voltage 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 reused 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.

Duration of blotting	**EVS1100**	**EVS1200**	**EV1300**
One hour	100 V 400 mA	100 V 400 mA	100 V 400 mA
Three hours	50 V 200 mA	50 V 200 mA	50 V 200 mA

Buffer solutions for blotting

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.

Towbin Buffer

- 25 mM Tris,
- 192 mM glycine,
- 20 % methanol pH 8.3

Towbin Buffer SDS

- 25 mM Tris
- 192 mM glycine
- 20 % methanol pH 8.3
- 0.05-0.1 % (w/v) SDS

Bjerrum and Schafer-Nielsen Buffer

- 48 mM Tris
- 39 mM glycine
- 20 % methanol pH 9.2

Dunn Buffer

- 10 mM $NaHCO_3$
- 3 mM $NaCO_3$
- 20 % methanol pH 9.9

Sequencing

Cleaning and preparation of glass plates

1. Better and more consistent results will be obtained if care is taken to ensure that the glass plates are as clean as possible. New glass plates must be cleaned in the same way as used glass plates because these will contain surface debris that may interfere with the gel.
2. First, clean using a neutral detergent and a small brush. Do not use metal wool or other test tube brushes, abrasive cleaning creams or scourers because these can scratch the surface of the glass plates.
3. The glass plates should be washed in the following sequence: distilled water ethanol, acetone, ethanol, distilled water. Thoroughly rinse and dry the glass plates before use. For extra clean plates, these should be wiped with a microscope tissue soaked in chloroform or dichloroethane in a fume hood.
4. To ease separation of the gel from the glass plates once the gel has been run, it is advisable to siliconise the notched glass plate with a tissue soaked in dimethyldichlorosilane. Wipe the plate, including the ears, in a fume hood. Rinse with water and dry with a tissue.
5. The plain glass plate should be siliconised along the outer 1 cm lengths where the spacers will be positioned. This should be periodically repeated when the gels start to stick to the plates. Plates should then be cleaned and siliconised again.
6. The horizontal gel pouring method described in this manual will not work if both plates are siliconised. In that case, use an alternative gel pouring method or do not siliconise the plain glass plate as described above.
7. The above procedures are not necessary every time a gel is poured.
8. After use, first, clean using a neutral detergent and a small brush and wash with distilled water, ethanol and acetone as described above.

Reagent preparation and gel volumes

1. For consistent gels it is advisable to use high quality reagents and where possible deionisine, degass and filtrate acrylamide gel solutions prior to use.
2. Made up acrylamide solutions should be stored in a refrigerator and allowed to reach room temperature prior to pouring.
3. It is always advisable to work using stock solutions which allow added convenience and save time when it comes to gel pouring. See the pages of stock solutions for SDS PAGE gels which should be pre-made beforehand. For DNA sequencing see the corresponding page. For native gel formulae and running conditions, please consult a laboratory manual.
4. As a guide, polymerisation conditions should be adjusted to effect polymerisation within about 5...15 minutes. Test a small volume in a vial prior to pouring the gel. As a rough guide 100 ml of degassed 6% acrylamide gel will set in about 5 minutes at room temperature when gently mixed with 450 µl of freshly prepared 10% (w/v) ammonium persulphate and 200 µl TEMED. The setting time increases

to about 10 minutes if the TEMED volume is reduced to 100 µl and to approximately 15 minutes with 75 µl. The amount of catalysts may need to be reduced under warm conditions.

5. Table below shows the volume of agarose solution required to make the desired gel (1 mm thick) for each unit size. Adjustments are needed for 0.75, 1.5 or 2 mm spacers.

	Gel size (cm)	Volume
ESEQ1100	33 x 45	125 ml

6. 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. See table below which details and which percentage of gel to use to separate the sizes of proteins indicated.

Acrylamide percentage	Separating resolution
5 %	60 – 220 kDa
7.5 %	30 – 220 kDa
10 %	20 – 75 kDa
12 %	17 – 65 kDa
15 %	15 – 45 kDa
17.5 %	12 – 30 kDa

For gel thicknesses thicker than 0.35 mm, first securely tape the bottom of the gel with electrical tape then follow the instructions below.

1. Lay the plain glass plate on a flat surface and arrange the spacers perfectly aligned with the edges of the plate.
2. Carefully place the notched glass plate on top of the plain glass plate and clamp the plates together using bulldog clips arranged along the edge of the glass plates, the pressure clamps of these should be in line with the spacers.
3. Fill a syringe with the required gel mix. Be careful not to agitate or to introduce bubbles into the solution.
4. Position the syringe above one edge of the notch in a vertical position. Steadily eject the gel solution along the notched area moving the syringe spout smoothly from one side of the notch to the other. The gel mix should form a continuous pool along the top of the gel space move down between the glass plates.
5. Be careful not to overfill the notched area. Fill gradually the gel solution should be around half the height of the notches on the notched glass plate. Also ensure not to under fill the notched area as air bubbles are more likely to be introduced between the glass plates. The boundary of the gel should

migrate as a straight line. To prevent or expel bubbles, the glass plates can be tapped lightly behind the moving gel boundary to prevent any bubble formation.

6. When the gel boundary reaches the bottom of the glass plates, remove all the surplus gel from the notched area with the syringe. This will ensure that the gel mix doesn't drip from the bottom of the glass plates.
7. Insert the comb. If a square well comb is used, insert the teeth making sure no bubbles are trapped. When using a shark's tooth comb, insert the flat face of the comb at a slight angle to prevent bubbles from being trapped. A few drops of gel mix can be added if necessary.
8. Carefully straighten the shark's tooth comb so that it is parallel to the top of the gel plate and reaches 3 - 5mm below the notched area.
9. For low percentage and DNA sequencing gels, leave to polymerise completely for at least 90 minutes. Low percentage gels can be left to polymerise overnight. To prevent the ends of the gel from drying out use wet tissues under a nesco film seal.

Assembling the unit

1. Insert the lower buffer chamber into position.
2. Remove the bulldog clips and the bottom tape, if used, from the glass plates.
3. Insert the glass plates behind the clamping bars and tighten the screws. Do not over-tighten the gel plate clamping screws as this may lead to the glass plate breakage and will also make the insertion and removal of combs difficult.
4. Attach the leads to the buffer chambers.
5. Ensure that the buffer drainage tap is in the closed position.
6. Into the upper buffer chamber pour between 400 ml min. and 1000 ml max. of electrophoresis running buffer.
7. Into the bottom buffer chamber pour between 400 ml min. and 1000 ml max. of electrophoresis running buffer. Important: do not fill over the maximum fill lines.
8. Prior to loading samples, flush out the wells with running buffer to clear them of urea and debris.

Preparation of samples for loading

DNA Sequencing

1. The volume of sample depends on the capacity of the wells (See Comb specifications page).
2. Heat the sequencing samples in a water bath or heating block at 95°C for 3 minutes, place on ice and centrifuge at 12000 rpm for 3 minutes. Return to ice.

Denatured protein

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 12000 rpm. The protein samples are now ready to load.

Gel running and ending

Running the gel

1. Remove the square tooth comb before loading the samples. If using a shark's tooth comb, leave this in position as the comb teeth will act as the wells.
2. Load the required volume of sample using a suitable loading tip. If possible, avoid taking the sample from the bottom of the tube (particulate materials may cause streaking or smearing). Sample dispersion can be minimized by loading the sample directly onto the bottom of the well and keep it as a thin layer.
3. Fit the safety lid ensuring it is positioned fully down over the electrical connectors.
4. Connect and run the gel at the desired power setting. The leads and electrical connectors are CE safe to 1500 V and users are advised not to exceed this voltage.
5. Typically for DNA sequencing gels, 45...55 W constant power is advised. For other types of gel please consult a laboratory handbook.
6. Turn the power supply off when the loading dye reaches the bottom of the gel, sooner if your proteins are below 4 Kd in size.

Ending the Run

1. **Disconnect and turn off the power supply before removing the leads.**
2. Remove the safety lid by gripping the edges of the lid and pushing down with your thumbs on the pegs located on the top of the unit.
3. Separate the plates with a strong, thin, broad blade. Do not force the glass plates apart at the notch as this may damage the 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. For protein gels, 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.

Solutions for DNA sequencing

10 x DNA sequencing Buffer

- 164.0 g Tris-OH
- 27.5 g boric acid
- 7.45 g disodium EDTA
- add distilled water to a final volume of 1000 ml

Acrylamide stock

- 38% acrylamide
- 2% bis-acrylamide

8% Gel

- 40.4 g urea
- 27.0 ml water
- 16.8 ml 38/2 acrylamide
- 8.0 ml 10 x DNA sequencing buffer

6% Gel

- 40.4 g urea
- 31.2 ml water
- 12.6 ml 38/2 acrylamide
- 8.0 ml 10 x DNA sequencing buffer

5% Gel

- 40.4 g urea
- 33.5 ml water
- 10.5 ml 38/2 acrylamide
- 8.0 ml 10 x DNA sequencing buffer

If necessary, mix by heating slightly, degassing is advised. Add 0.7 ml 10% ammonium persulphate, and 25 µl TEMED, and pour gel immediately.

Troubleshooting

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. Add endonucleases (for each benzonases). Precipitate protein with TCA/acetone to diminish carbohydrate content.
Problem: Gel casting and sample loading	**Cause**	**Solution**
Poor well formation	Incorrect catalyst used Monomer solution not degassed (oxygen inhibits polymerization)	Prepare Fresh catalyst solution. Increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED. Degas monomer solution immediately prior to casting stacking gel.
Webbing; excess acrylamide behind the comb	Incorrect catalyst concentration	Prepare fresh catalyst solution. 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 Failure to degas Temperature too low Poor quality acrylamide or bis Old APS	Use 0.0.05% APS and 0.05% TEMED. Degas monomer solutions 10-15min. Cast at room temperature, warming glass plates if necessary. Use electrophoreses-grade reagents Prepare fresh APS.
Swirls in the gel	Excess catalysts; polymerization time < 10min Gel inhibition; polymerization time >2hr	Reduce APS and TEMED by 25% each. Increase APS and TEMED by 50%; degas.
Gel feels soft	Low %T Poor quality acrylamide or bis Too little cross-linker	Use different %T. Use electrophoresis- grade reagents. 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 Pipetting, loading error	Induce 10% glycerol in sample to make it denser than surrounding buffer. 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 Insufficient buffer in inner buffer chamber Insufficient buffer in outer buffer chamber Electrical disconnection	Remove tape. Fill buffer chamber with running buffer. Fill inner and outer buffer chambers to ensure wells are completely covered. 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 Running or reservoir buffer too dilute Voltage too high	Check buffer composition and type. Check buffer protocol and concentrate if necessary. Decrease voltage by 25-50%.
Gels run slower than expected	Incorrect running buffer composition or type Excessive salt in sample	Check buffer composition and type. Desalt sample.
Buffer leaking from inner chamber	Incomplete gasket seal	Set up again with sliding clamps tighter.
Problem: Total Protein Staining	**Cause**	**Solution**
Bands not visible	No protein in gel Imaging system malfunctioning Incorrect imaging parameters were used	Stain with another method to confirm there is protein. Check instrument manual for troubleshooting or contact imaging instrument manufacturer. Check Instrument manual.
Poor staining sensitivity	Dirty staining trays Insufficient stain volume Insufficient staining time Reuse of staining solution	Clean staining trays and other equipment with laboratory glassware cleaner. Follow recommendations for stain volume (appropriate to gel size). Increase staining time. Repeat staining protocol with fresh staining solution.
High or uneven background staining	Staining trays or equipment dirty Too much time in staining solution Reagent impurities	Clean staining trays and other equipment with laboratory glassware cleaner. Restrict duration of incubation in staining solutions as recommended in protocol. Wash gel in water or retrospective destaining solution for >30min. 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. Decrease time that gels and staining solution are exposed to open air. Use dust-free gloves and handle gels only by edges.

Uneven staining	Insufficient shaking during staining	Agitate gel during staining.
Gel shrinkage	Gel dehyrated	Transfer gel to water for rehydration.
Problem: Evaluation of Separation	**Cause**	**Solution**
Diffuse or broad bands	Poor quality acrylamide or bis-acrylamide incomplete polymerization Old SDS or sample buffer Gel temperature too high	Use electrophoresis-grade reagents. Check polymerization conditions. Prepare fresh solutions. 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 Power conditions excessive Insufficient buffer	Check buffer composition; buffer not mixed well, or buffer in upper chamber too concentrated. Prepare new buffer, ensuring thoroughly mixing, especially when diluting 5x or 10x stock. 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. Fill inner and outer buffer chambers to ensure that wells are completely covered.
Smiling or frowning bands with gel lane	Overloaded proteins Sample preparation/ buffer issues Incorrect running conditions	Load less protein. Minimize salts, detergents and solvents in sample preparation and sample buffers. Use correct voltage.
Skewed or distrorted bands, lateral band spreading	Excess salt in samples Ionic strength of sample lower than that of gel Insufficient sample buffer or wrong formulation Diffusion prior to turning on current Diffusion during migration through stacking gel Uneven gel interface	Remove salts, from sample by dialysis or desalting column prior to sample preparation. Use same buffer in samples as in gel. Check buffer composition and dilution instructions. Minimize time between sample application and power startup. Increase %T of stacking gel to 4.5% or 5%T. Increase current by 25% during stacking. Decrease polymerization rate. Overlay gels carefully. Rinse wells after removing comb to remove residual acylamide.
Vertical streaking	Overloaded samples Sample precipitation	Dilute sample. Selectively remove predominant protein in sample (fractionate). Reduce voltage by 25% to minimize streaking. Centrifuge samples to remove particulate prior to sample loading.

		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.

Certificate

CE

EU

DECLARATION OF CONFORMITY

We declare under our sole responsibility that the products

Horizontal and Vertical Electrophoresis units

content of the type numbers

EHS1050, EHS1100, EHS1200, EHS1300, EHS1400, EHS1500,

EHS3100, EHS3200, EHS3300, EHS3350, EHS3400, EHS3410, EHS3500, EHS3600, EHS3610, EHS3620, EHS3660,

EVS1100, EVS1200, EVS1300,

EVS3100, EVS3200, EVS3300,

to which this declaration relates is in conformity
with the following standards of the European Directives

LOW VOLTAGE DIRECTIVE 2014/35/EU
EN61010-1: 2010

ROHS DIRECTIVE 2011/65/EU
EN50581: 2012

Name Jan De Ceuster
Title Manager
Date 27 May 2016

Signature

Consort bvba
Hertenstraat 56 unit 9, B-2300 Turnhout, Belgium
Tel: (+32) (0)14 41 12 79
Fax: (+32) (0)14 42 91 79
Sales: sales@consort.be

Warranty

The **Consort** Horizontal 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, **Consort** 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 **Consort** 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 **Consort** or its associated distributors have invalidated warranty.

Consort 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.

Consort products are for research use only. **Consort** is not liable for consequential damages arising out of the use or handling of its products.

A return authorisation must be obtained from **Consort** before returning any product for warranty repair on a freight prepaid basis. If a problem does occur then please contact your supplier or **Consort**:

Consort bvba
Hertenstraat 56/9
2300 Turnhout
Belgium

Tel: +32 (0)14 41 12 79
Email: info@consort.be

Record the following for your records:

Model _____

Date of Delivery _____

Warranty Period _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

A return authorisation must be obtained from **CONSORT** before returning any product for warranty repair on a freight prepaid basis!

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