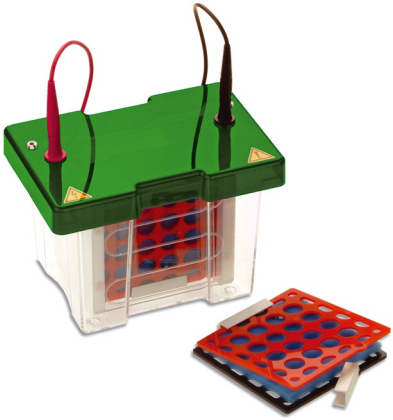
Consort

MANUAL



E5100
E5200
E5300
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E5700

July 2009

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CONSORT guarantees that the unit you have received has been thoroughly tested and meets its published specification.

This unit (excluding all accessories) is warranted against defective material and workmanship for a period of twelve (12) months from the date of shipment ex factory.

CONSORT will repair all defective equipment returned during the warranty period without charge, provided the equipment has been used under normal laboratory conditions and in accordance with the operating limitations and maintenance procedures outlined in this instruction manual and when not having been subject to accident, alteration, misuse or abuse.

No liability is accepted for loss or damage arising from the incorrect use of this unit. **CONSORT**'s liability is limited to the repair or replacement of the unit or refund of the purchase price, at **CONSORT**'s option. **CONSORT** is not liable for any consequential damages.

CONSORT reserves the right to alter the specification of its products without prior notice. This will enable us to implement developments as soon as they arise.

CONSORT products are for research use only.

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

- 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 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.

- 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 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 (H_2O_2) 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.

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”.

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.

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 manufacturers 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 / **ml**	**E5100**	**E5200**	**E5300**
One cassette	1380	2800	5600
Two cassettes	1290	2620	5240
Three cassettes	1200	2440	4880
Four cassettes	1110	2260	4520

Each cooling pack will take the place of 100 ml of buffer for E5100 and 500 ml of buffer for E5200 and E5300.

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 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.

Duration of blotting	**E5100 E5200**	**E5300**
One hour	100 V	100 V
	400 mA	400 mA
Three hours	50 V	50 V
	200 mA	200 mA

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

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.

Setting up the blot sandwich

Please consult the solutions for details on buffers required for protein, DNA or RNA blotting. The recommended buffers and alternatives are given further in this manual. This also provides a guide for the amount of standard grade filter paper pieces required for each buffer. Adjust amounts accordingly for thicker grade filter paper.

1. Cut the membrane and filter paper to the size of the gel. **At no point touch the membrane with bare fingers as this will cause changes in the surface properties of the membrane and cause inconsistent sample binding.**
2. Depending on the type of membrane used, the membrane may need equilibrating in buffer prior to blotting. This is generally true of PVDF and nylon membranes but varies for nitrocellulose membranes. Please consult the membrane manufacturer's guidelines for wetting the membrane prior to blotting.
3. Ensure that excess liquid drains from the membrane.
4. Soak the required number of filter paper pieces in the appropriate buffer. Generally six filter paper pieces are required per electrode, twelve in total.
5. Mark or use some other means to enable identification of the gel side of the membrane. This is necessary for the blot probing stage, more efficient binding can occur when the membrane is facing upwards in the probe solution. It is also an idea to clip the corner of the gel nearest to the top of lane 1 was on the gel to allow easy sample identification during analysis.
6. Remove the lid from the blotter and place six pre-soaked filter paper pads onto the base electrode plate ensuring that any excess liquid is wiped away.
7. Carefully place the membrane on top of the six filter paper pads and ensure that no air pockets have formed. Any air pockets should be smoothed out using a wet gloved finger.
8. Place the gel on top of the membrane and smooth to ensure no air pockets have formed. It may help to add a small amount of transfer buffer to the gel to help the membrane attach to the gel evenly.

9. Place the remaining six filter paper pads on top of the membrane and gently smooth.
10. Carefully place the lid over the blot sandwich and secure using the screws. These should be tightened evenly a little each at a time. The blot may de disturbed if one screw is tightened fully, then the next. **Note: only use the screws for blotting of acrylamide gels up to 2 mm thick. For blotting thicker gels and agarose gels do not use the screws. The weight of the lid will provide enough pressure or a small container of buffer 0.5...1 litre container can be used as a weight**
11. Connect the leads to the unit, red to the positive base and black to the negative lid. **Note: The red lead inserts through the lid into the base. The black lead inserts through the side of the base into the lid. This is a necessary safety feature so that the electrodes cannot be accessed when the unit is connected to a power supply.**
12. Attach the power leads to the appropriate sockets, red to red, black to black on a power supply. **Do not invert the leads or connect up incorrectly as this will cause corrosion of the stainless steel electrode.**
13. Blotting generally requires high current settings >250 mA and the power supply should contain these capabilities. Please see the Consort range for details of these.

1. For the E5400, set the power supply to run at 80...200 mA, 10...15 V depending on the time you want to transfer. For the E5500, set to 320...800 mA, 15...25 V. Similar settings are advised for the E5600 and E5700 units though a time course study is highly recommended to establish the most efficient settings. Higher current settings may cause excessive heat generation and the following upper limits must never be exceeded:

   E5400: 550 mA, 75 V

   E5500: 1200 mA, 75 V

   E5600: 2000 mA, 75 V

   E5700: 2000 mA, 75 V

2. Allow transfer to proceed for between 30 minutes to one hour depending on current settings.

3. The efficiency and quality of transfer depends on the type of buffer used and also the type of samples. Generally, better results can be obtained by reducing the power settings and increasing the blot time. It is generally best to do a time course for the type of gels, samples and buffers being used to ensure that transfer occurs at maximum efficiency.

Transfer buffers must be made accurately using high grade reagents. Do not adjust the pH with acid or base as this will affect the properties of the buffer. pH will vary according to the purity of the reagents used.

Triple buffer system

- For high efficiency transfer of Protein from acrylamide gels
- Anode 1 Buffer: 0.3 M Tris base, 20% MeOH, pH 10.4: soak 4 standard grade filter paper sheets
- Anode 2 Buffer: 0.025 M Tris base, 20% MeOH pH 10.4: soak 2 standard grade filter paper sheets
- Cathode Buffer: 0.025 M Tris base, 0.04 M Caproic Acid, 20% MeOH pH 9.4: soak 6 standard grade filter paper sheets

DNA (Southern) Blotting Buffer

- For high efficiency transfer of DNA from agarose gels.
- Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:

  50 x 1 M ethanolamine-glycine buffer, pH 11

RNA (Northern) Blotting Buffer

- For high efficiency transfer of RNA from agarose gels.
- Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:

  50 x 0.2 M morpholinopropanesulfonic acid (MOPS)

  50 mM sodium acetate

  5 mM EDTA

  pH 7.0

Nucleic acid transfer buffers

Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:

- 1 x TAE 40 mM Tris (pH 7.6), 20 mM acetic acid, 1 mM EDTA
- 50 x (1l) dissolve in 750 ml distilled water:
  242 g Tris base (FW = 121)
  57.1 ml glacial acetic acid
  100 ml 0.5 M EDTA (pH 8.0)
- Fill to 1 litre with distilled water.

Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:

- 1 x TBE 89 mM Tris (pH 7.6), 89 mM boric acid, 2 mM EDTA
- 10 x (1l) dissolve in 750 ml distilled water:
  108 g Tris base (FW = 121)
  55 g boric acid (FW = 61.8)
  40 ml 0.5 M EDTA (pH 8.0)
- Fill to 1 litre with distilled water.

Transfer buffers

Towbin Buffer with 20% methanol

Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:

- 0.025 M Tris base
- 0.192 M glycine
- 20% methanol.
- pH 8.3

DECLARATION OF CONFORMITY

We declare under our sole responsibility that the product

Electrophoresis Apparatus
content of the type numbers

E5100, E5200, E5300, E5400, E5500, E5600, E5700

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

EN61010
LOW VOLTAGE DIRECTIVE 73/23/EEG
EN50081-1
EN50082-1
EN60555-2
EMC DIRECTIVE 89/336/EEG

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The electro-magnetic susceptibility has been chosen at a level that gains proper operation in residential areas, on business and light industrial premises and on small-scale enterprises, inside as well as outside of buildings. All places of operation are characterised by their connection to the public low voltage power supply system.

Consort

www.consort.be

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