SIGMA-ALDRICH

INSTRUCTION MANUAL

ELECTRO-BLOTTING UNIT
Z34,046-4 Wet Blotting Module
Z34,047-2 Wet Blotting Set

WARNING

THESE UNITS ARE CAPABLE OF DELIVERING POTENTIALLY LETHAL VOLTAGE WHEN CONNECTED TO A POWER SUPPLY AND ARE TO BE OPERATED ONLY BY QUALIFIED TECHNICALLY TRAINED PERSONNEL.

PLEASE READ THE ENTIRE OPERATOR'S MANUAL THOROUGHLY BEFORE OPERATING THIS UNIT.

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

THE SIGMA ELECTRO-BLOTTING UNITS ARE DESIGNED TO GIVE LONG SERVICE AND REPRODUCIBLE RESULTS IN YOUR LABORATORY. A FEW MOMENTS SPENT READING THESE INSTRUCTIONS WILL ENSURE THAT YOUR EXPECTATIONS ARE REFLECTED IN THE SUCCESSFUL USE OF THE APPARATUS.

FIRST CHECK THAT THE APPARATUS HAS BEEN RECEIVED COMPLETE AND UNDAMAGED FOLLOWING SHIPMENT. ANY FAULTS OR LOSSES MUST BE NOTIFIED TO SIGMA-ALDRICH IMMEDIATELY, SIGMA-ALDRICH CANNOT ACCEPT RESPONSIBILITY FOR GOODS RETURNED WITHOUT PRIOR NOTIFICATION.
REFER TO THE PACKING LIST AND CHECK THAT ALL COMPONENTS AND ACCESSORIES ARE PRESENT.

PLEASE RETAIN ALL PACKAGING MATERIALS UNTIL THE WARRANTY PERIOD HAS EXPIRED.

SPECIFICATIONS:

Z34,046-4 is a wet blotting module, with cassettes & fibre pads, designed for use with the tank, safety lid and cables from the Sigma Cooled Mini Vertical Gel Electrophoresis set.

Z34,047-2 is a ready-to-use set of wet blotting module, with cassettes and fibre pads, tank, safety lid and cables.

Construction:

- Rugged acrylic construction.
- All acrylic joints chemically bonded.
- Doubly insulated cables, rated safe up to 1,000 volts.
- Gold plated electrical connectors, corrosion-free and rated safe up to 1,000 volts.
- Recessed power connectors, integral with the safety lid.
- 0.2mm diameter platinum electrodes, 99.99% pure.
- User replaceable platinum electrode cassettes.
- Compression cassettes with an open square grid design, which provides a maximum area for transfer.
- Simple compression cassette locking mechanism

Environmental Conditions

- This apparatus is intended for indoor use only.
- This apparatus can be operated safely at an altitude of 2,000m.
- The normal operating temperature range is between 4^0C and 65^0C.
- Maximum relative humidity 80% for temperatures up to 31^0C decreasing linearly to 50% relative humidity at 40^0C.

- 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”.

Operational:

Overall Dimensions (WxLxH)	12 x 13 x 21cm
Approx. Total Buffer Volume (L)	1L
Electrode separations	8cm
No. of Gel Cassette Slots	4
No. Electrode Coils per Face	6 (1.5cm apart)
Approx. Electrode length per Face	68cm
Approx. Gel Sizes:	up to 8.5 x 9.5cm
Max. Operating Voltage	500 Volts
Max. Operating Current	2 Amps
Electrical Connectors	4mm male fully shrouded.

USING THE ELECTRO-BLOTTING UNITS

A. Safety Precautions

- **READ** the instructions before using the apparatus.
- Always isolate the units from their power supply before removing the safety lid. Isolate the power supply from the mains **FIRST** then disconnect the leads.
- **DO NOT** exceed the maximum operating voltage or current (see Specifications).
- **DO NOT** operate the units in metal trays.
- Acrylamide is a volatile, cumulative neurotoxin and suspected carcinogen. Wear effective clothing and follow the recommended handling and disposal procedures.
- Polymerised gels contain some unpolymerised monomer. Handle only with gloves.
- Following the replacement of a platinum electrode, have the unit inspected and approved by your safety officer prior to use.
- **DO NOT** fill the units with transfer 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 being employed. To disperse these gases make sure that the apparatus is run in a well ventilated area.

B. General Care and Maintenance

- To remove the safety lid, push down on the plastic lugs and lift the lid vertically with your fingers.
- Before use clean and dry the apparatus with **DISTILLED WATER ONLY.**
  IMPORTANT: Acrylic plastic is NOT resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohols (over 25%) and acids (over 25%).
- Before use, and then on a monthly basis, check the unit for any leaks at the chemically bonded joints. Place the unit on a sheet of dry tissue paper and then fill with **DISTILLED WATER ONLY**, to the maximum fill line. Any leakage will be seen on the tissue. If any leakage is seen, **DO NOT ATTEMPT TO REPAIR OR USE THE APPARATUS**, but notify SIGMA immediately.
- When cleaning the replacement platinum electrodes DO NOT use cleaning brushes. Usually, a thorough rinse with distilled water is all that is required.
- Ensure that the connectors are clean and dry before usage or storage.

C. Operating Instructions:

- Cool and degas the appropriate volume of transfer buffer (see specifications). The buffer should be degassed prior to the addition of SDS. The most commonly used transfer buffer is:

25mM Tris, 192mM glycine, 20% methanol pH8.3, Ref. Towbin et al (1979).
This buffer can be used with or without 0.05-0.1% (w/v) SDS.

Other transfer buffers include:

1. 48mM Tris, 39mM glycine, 20% methanol pH9.2, Ref. Bjerrum and Schafer-Nielsen (1986)

2. 10mM $NaHCO_3$, 3mM Na_2CO_3, 20% methanol pH9.9, Ref. Dunn (1986).

- Half fill the tank with transfer buffer.
- Connect the cooling water supply to the tank base hose connectors. Use hose clips to secure the tubing.
- Add a magnetic flea to the tank to maintain uniform temperature and conductivity during electrophoretic transfer.
- Use gloves when handling the compression cassette components described below.
- Pre-equilibrate the gel in cool transfer buffer to remove SDS and salts. This serves to prevent the gel changing size during transfer and to reduce heating effects.
- Soak a gel-sized piece of transfer membrane in transfer buffer for 15 minutes.
- Cut four pieces of laboratory grade blotting paper to a size 5mm larger than the gel to be blotted. Soak them in transfer buffer.
- Soak the fibre pads in transfer buffer. If the pads are larger than the gels you routinely use they can be trimmed down with a pair of scissors.
- Familiarise yourself with the interlocking hinge of the compression cassette.
- Carefully align and overlay the transfer membrane onto the gel in one smooth action. Work from the centre and let the ends progressively roll down. Do not attempt to reposition the membrane as some transfer may occur on contact. Use a clean glass rod wetted in transfer buffer to roll out any trapped bubbles.
- Assemble the gel/membrane sandwich in the compression cassette as described below and as illustrated in figure 1. Note the orientation of the gel relative to the membrane and use a pencil or marker pen (on the clamp) to mark the +ve side (i.e. membrane side) of the cassette.
- Finally, gently squeeze the cassette sides together and slide the clamping clip onto the top edge.

- Insert the cassette into one of the tank slots with the transfer membrane on the +ve side.
- Add further transfer buffer until the top loops of the platinum coils are just covered.
- Apply the safety lid and refer to Table 2 for operating conditions. Note that operating conditions should be optimised for your applications. Factors that will affect the success of transfer include: gel porosity, buffer composition and pH, transfer time, transfer field density (V/cm), molecular weight range, stirring of buffer and temperature control, choice of membrane and the detection system chosen. Residual salts in the gel could cause the system to heat up so pre-equilibration of the gel with transfer buffer is important.
- Initially, chose a buffer system such as that of Towbin et al (1979) and perform a pilot time course experiment, transferring molecular weight markers from your gel to the membrane of your choice. It is better to start with low current or voltage setting, monitoring the temperature as the run proceeds. If the temperature is controlled and transfer is successful you could try higher settings for subsequent runs.

Table 2

Transfer Buffer *	**Overnight**	**1 hour**
Towbin Buffer	25 - 40 V 40 - 80mA	50 - 100 V 200 - 400mA
Bjerrum Buffer	25 - 40 V 40 - 80mA	50 - 100 V 200 - 400mA
Dunn Buffer	10 V 40 - 80mA	40 - 80 V 200 - 500mA

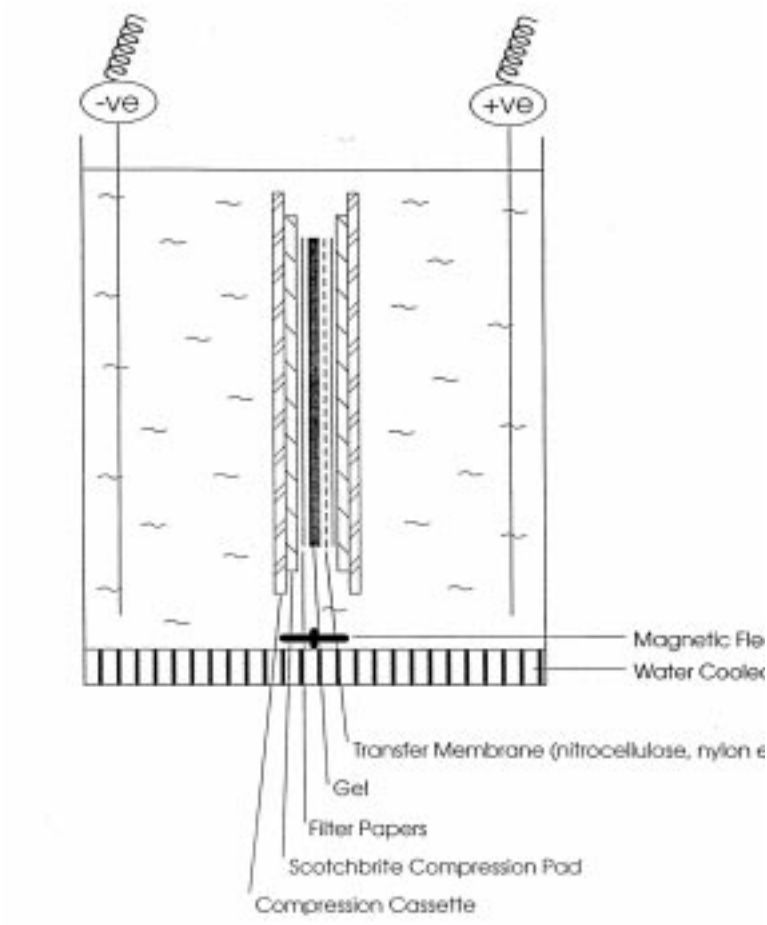
* Do not pH with acid or alkali. The pH may vary slightly according to the purity of reagents and accuracy of weighing. Adding ions may cause overheating during transfer.

- If an acidic buffer is chosen, e.g. 0.7% acetic acid the direction of transfer will be the opposite of normal, i.e. transfer will be from anode to cathode. Such a buffer would be suitable for IEF gels or basic protein gels (native).

Assemble in the order:

1. Cassette clamp -ve side.
2. Pre-wetted pad.
3. 2 pre-wetted blotting papers.
4. Gel
5. Pre-wetted transfer membrane.
6. 2 pre-wetted blotting papers.
7. Pre-wetted pad.
8. Cassette clamp +ve side.

Figure 1. Assembly of the gel/membrane sandwich in the compression cassette.



D. Cleaning and Storage

After use, thoroughly rinse all components in deionised water and gently dry the gold electrode connectors with a soft tissue. **NEVER USE ORGANIC SOLVENTS.**

E. References

1. Towbin, J., Staehelin, T., and Gordon, J. (1979). Electrophoetic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications, Proc. Natl., Acad. Sci. USA, 76, 4350-4354.

2. Bjerrum, O.J. and Schafer-Nielsen, C. (1986). Buffer systems and transfer parameters for semi-dry electroblotting with horizontal apparatus, in Dunn Electrophoresis 1986, 315-327, VCH, Weinheim, Germany.

3. Dunn, S.D. (1986). Effects of the modification of transfer buffer composition and the renaturation of proteins in gels on the recognition of proteins on Western blots by monoclonal antibodies, Anal. Biochem. 157, 144-153.

F. Further Reading

B.D. Hames (1990) Blotting Techniques Ch.1, 7.10, p. 85-97. In: Gel Electrophoresis of Proteins, A Practical Approach, 2nd Edition, B.D.Hames and D.Rickwood, eds., IRL Press.

Related Products

The following Sigma-Aldrich chemical and other consumable products are mentioned in this manual.
For up-to-date packs and prices, see www.sigma-aldrich.com or contact your local Sigma-Aldrich sales office (see back of manual)

Related product	Sigma-Aldrich Prod. No.
Tris	T1503
Glycine	50050
Methanol	M1770
Sodium carbonate	S7795
Sodium bicarbonate	S6297
SDS	71729
Nylon membranes	N1031 and N4781
Blotting paper (Whatman 3MM Chr)	Z27,090-3

PACKING LIST Z34,046-4

No. Items	Description	Replacement Part Number	Check
1	Blotting module	-	
4	Compression Cassettes	Z340480	
2	Fibre Pads, 10 x 10cm, PK/4	Z340499	

PACKING LIST Z34,047-2

No. Items	Description	Replacement Part Number	Check
1	Tank & safety lid with cables	-	
1	Blotting module	-	
4	Compression Cassettes	Z340480	
2	Fibre Pads, 10 x 10cm, PK/4	Z340499	

QUALITY CHECK LIST

Model..... Serial Number.....

1. Tank Leak Tested Check.....
2. Electrode Conductivity Test Check.....
3. Labels Positioned Check.....
4. Labels Test/Serial No. Check.....
5. Unit Scratch/Blemish Free Check.....
6. Accessories - See Packing List Check.....
7. Instructions Check.....

ALL SIGMA PRODUCTS ARE SUPPLIED HAVING PASSED RIGOROUS QUALITY CONTROL PROCEDURES.
IF YOU HAVE A QUERY, CONTACT YOUR LOCAL SIGMA-ALDRICH SALES OFFICE FOR TECHNICAL SUPPORT.

SIGNED..... QUALITY CONTROL ASSESSOR

WARRANTY

SIGMA-ALDRICH 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.

SIGMA-ALDRICH 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. SIGMA-ALDRICH's liability is to the repair or replacement of the unit or refund of the purchase price, at SIGMA-ALDRICH's option. SIGMA-ALDRICH is not liable for any consequential damages.

SIGMA-ALDRICH 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.

SIGMA-ALDRICH products are for research use only.

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

WARNING

DO NOT attempt to remove the outer casing or make repairs to our electrical range of products, should any unit fail.
Contact SIGMA-ALDRICH immediately if the need for repair or servicing should arise.

See back cover for contact details of your local Sigma-Aldrich office

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