

Product Information

Sigma-Aldrich® SB10 omniPAGE mini Electroblotting System - Complete Electroblotting Unit

Catalog Number **EP1501**
 Store at Room Temperature
 Technical Bulletin AL-262

TECHNICAL BULLETIN

Product Description

The Sigma-Aldrich® SB10 omniPAGE mini Electroblotting System is a stand-alone, complete unit for electroblotting. This system includes tank, lid, electroblotting module, cassettes, and fiber pads. In addition, hinged rigid cassettes allow rapid set up and convenience to ensure that even contact between the gel and membrane is maintained.

The SB10 omniPAGE mini electroblotting system (EP1501) may be combined with the CVS10DSYS omniPAGE mini vertical gel electrophoresis unit (EP1300) to create a complete vertical gel electrophoresis and electroblotting system.

Guidelines and Restrictions

- Maximum altitude – 2,000 m (6,562 ft)
- Temperature range between 4–65 °C
- Maximum relative humidity 80% for temperatures up to 31 °C, decreasing linearly to 50% relative humidity at 40 °C.
- Not for outdoor usage

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

Components

System includes tank, lid, electroblotting module, electrodes, and accessories:

Table 1.

Accessories included with SB10 omniPAGE mini Electroblotting System

Product	Blotting Insert	Cassettes	Fiber Pads
SB10 Electroblotting System (EP1501)	EP1502: 10 × 10 cm Mini Blotting Insert	EP1503: 10 × 10 cm mini cassette × 3	EP1504: 10 × 10 cm fiber pads (2 packs of 6)

Additional components are available (see Appendix).

Refer to the packing lists as soon as the unit is received to ensure all components have been included. The system should be checked for damage when received. Please contact Sigma-Aldrich if there are any problems or missing items.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Please read the entire technical bulletin before using these units.

Preparation Instructions

Setting up the Tank - 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 is positive.
2. Remove the lid from the unit.
Note: If the lid is not removed, fitting the cables may result in loosening the gold plug and damage to the electrode.
3. Screw the cables into the tapped holes as fully as possible, so there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

Electroblotting Buffer Solutions

Note: Do not adjust the pH when making these buffers as this will cause blot overheating. The pH will vary according to the freshness of the reagents used.

- A. Towbin Buffer (25 mM Tris, 192 mM glycine, and 20% methanol, pH 8.3)
- B. Towbin Buffer with SDS (25 mM Tris, 192 mM glycine, and 20% methanol, pH 8.3, with 0.05–0.1% (w/v) SDS)
- C. Bjerrum and Schafer-Nielsen Buffer (48 mM Tris, 39 mM glycine, and 20% methanol, pH 9.2)
- D. Dunn Buffer (10 mM NaHCO₃, 3 mM Na₂CO₃, and 20% methanol, pH 9.9)

Storage/Stability

The units may be stored at room temperature and operated in the temperature range of 4–65 °C.

Procedure

Protein Blotting using the Electroblotter

A. Setting up the Blot Sandwich

Note: For the most commonly used electroblotting buffer solutions see Preparation Instructions, Electroblotting Buffer Solutions.

1. Each blot sandwich should be set up as follows:
 - a. Cassette clamp -ve (negative electrode, black) side placed in a tray or other suitable surface.
 - b. Presoaked fiber pad.
Note: two fiber pads can be used with thin gels.
 - c. Two pieces of thick filter paper, 2–3 mm thick, presoaked in buffer.
 - d. PAGE gel
 - e. Transfer membrane – Usually the transfer membrane requires presoaking but consult with the manufacturer's instructions for the type of membrane used. This should be smoothed so that no air bubbles have been trapped.
 - f. Two pieces of thick filter paper, 2–3 mm thick, presoaked in buffer.
 - g. Presoaked fiber pad.
Note: two fiber pads can be used with thin gels.
 - h. Cassette clamp +ve (positive electrode, 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 electroblotting buffer solution up to the **maximum fill line** indicated on the side of each unit. See Table 2 for buffer volume required.
Note: Improved transfer can usually be obtained by using chilled buffer.

Table 2.

Buffer volume for the Electroblotter Module

Note: Each cooling pack will take the place of 100 mL of buffer.

Number of Cassettes	Buffer Volume
1 Cassette	1,380 mL
2 Cassettes	1,290 mL
3 Cassettes	1,200 mL

B. Running the Blot

1. Insert the cassettes into the slots in the electroblotting 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. This can be done by noting which samples were loaded adjacent to each respective 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 center 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 necessary to further aid cooling.
3. Insert the module, fit the lid, and connect to a power supply.
4. Consult Table 3 for recommended power supply voltage settings and blot times. Please note voltages and current will vary according to the number of cassettes, type and temperature of buffer, and thickness and percentage of gel. These will also affect quality of transfer, so the time course of transfer should be performed for the particular samples and conditions.

Table 3.
Recommended Voltages and Average Resultant Currents

Duration of Blotting	omniPage mini electroblotter module
1 Hour	100 V 400 mA
3 Hours	50 V 200 mA

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.

References

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

Trizma is a registered trademark of Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co
Parafilm is a registered trademark of American Can Co.

MG,MAM 06/10-1

Appendix

Table 4.
Additional Equipment

Product Description	Catalog Number
Parafilm [®] M roll size 2 in. × 250 ft	P7543
Parafilm M roll size 4 in. × 125 ft	P7793
Parafilm M roll size 4 in. × 250 ft	P7668
Screws × 4	EP1306
Cables (Pack of 2)	EP2002
10 × 10 cm mini cassette × 3	EP1503
10 × 10 cm fiber pads 2 packs of 6	EP1504
Cooling Packs	EP1311
Gel Staining Tray	T0567
Transilluminator UV/White light AC input 115 V, 60 Hz	Z363820
Power supply 250V AC input 110 V US 3-pin plug	PS2501
Power supply 250V AC input 220 V EU 2-pin plug	PS2502

Table 5.
Electroblotting Chemicals

Product Description	Catalog Number
Trizma [®] Base, primary standard and buffer, >99.9% (titration)	T1503
Glycine, for electrophoresis ≥99%	G8898
Methanol, BioReagent,	M1770
Sodium Dodecyl Sulfate (SDS), for electrophoresis, ≥98.5% (GC)	L3771
Sodium Bicarbonate (NaHCO ₃), BioXtra ≥99.5%	S6297
Sodium Carbonate (Na ₂ CO ₃), BioXtra ≥99.0%	S7795
Water, Molecular Biology Reagent	W4502

Aldrich brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.