
Instruction manual

PerfectBlue™ 'Semi-Dry' Electro Blotter

Sedec™ S & Sedec™ M



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WARRANTY

PEQLAB guarantees that the semi-dry electro blotting system you have received has been thoroughly tested and meets its published specification.

However, immediately upon arrival, please check carefully that the shipment is complete and has not been damaged in transit. For missing parts or to report any kind of damage, please contact PEQLAB (see 'TECHNICAL SUPPORT AND ORDERING INFORMATIONS'). Please retain all packaging materials until the delivery has been completely checked since this will speed up the return of goods if required and reduce environmental impact. Any form of returns, replacements or credit notes must be agreed in advance by PEQLAB.

For the complete range of PerfectBlue™ electrophoresis and blotting systems, PEQLAB guarantees a warranty period of 36 months if the products have been used solely according to the instruction manual, unless a different warranty has been offered in writing. After the warranty period has expired, PEQLAB can offer repairs. No liability is accepted for loss or damage arising from incorrect use. PEQLAB's liability is limited to the repair or replacement of the unit or refund of the purchase price, at PEQLAB's discretion. PEQLAB is not liable for any consequential damages.

PEQLAB reserves the right to alter the technical specifications of the PerfectBlue™ electrophoresis or blotting systems without prior notice. This will enable us to implement developments as soon as they arise.

PACKAGING LIST

Unless requested otherwise, the following items are included in shipment for the models Sedec™ S and Sedec™ M:

- one base with platinum-plated titanium anode
- one safety lid with stainless steel cathode
- one pair of power cords
- three screwing knobs for lid fixation
- one instruction manual

SAFETY PRECAUTIONS

- Please read this Instruction Manual carefully before using the blotting system.
- Only use a CE marked DC power supply.
- Always disconnect the system from the power supply before removing the safety lid.
- Always disconnect the system from the power supply when it is not in use or before moving it.
- Running conditions for this unit should not exceed the maximum operating voltage or current.
- Do not use the system if any part is broken.
- Connect the stainless steel electrode plate of the lid with the negative pole of the power source only. It will corrode otherwise.

SYSTEM OVERVIEW

PerfectBlue™ Semi-Dry Electro Blotting Systems offer rapid transfer of proteins or nucleic acids from polyacrylamide or agarose gels to membranes. Solid and highly conductive electrode plates made of platinum-plated titanium (anode) or stainless steel (cathode) allow transfer at low voltages without external cooling systems. In addition, they provide an exceptional uniformity of the electrical field and by doing so transfer results of highest quality.

The semi-dry Blotter Sedec™ S has a transfer area of 10 x 10 cm and fits the typical mini gel format. The model Sedec™ M offers a large transfer area of 20 x 20 cm for the simultaneous transfer of several gels or for the transfer of large gels.

Because of its weight, the solid safety lid assures maximal and even electrical contact between electrode plates and blotting sandwich. Its safe fixation is realized fast and comfortably by using three handy and smooth-running screwing knobs.

For Semi-Dry Electro Blotting systems Sedec™ S and Sedec™ M, the anode plate (positively charged electrode) is located in the base while the cathode plate (negatively charged electrode) is located inside the safety lid. Therefore negatively charged target molecules (usually proteins or nucleic acids) will get transferred top down.

Technical properties

PerfectBlue™	Cat. No.	Transfer area	Buffer volume	Current	typ. transfer time
Sedec™ S	52-1010	10 x 10 cm	ca. 50 ml*	50-300 mA	30-120 min
Sedec™ M	52-2020	20 x 20 cm	ca. 200 ml*	200-1200 mA	30-120 min

* for soaking blotting papers and membranes

GENERAL INSTRUCTIONS

Materials required

To begin, prepare buffers, blotting papers and membranes needed for electro blot transfer. Cut filter paper and transfer membrane to match the size of the gel and saturate several sheets of filter paper in transfer buffer(s). Filter paper must be thoroughly saturated with transfer buffer. In general, three sheets of filter paper are used on each side of the stack. The transfer membrane preparation will depend on the type of membrane used. Refer to manufacturer's instructions for this information. In some cases, it is recommended that the gel is soaked in transfer buffer before blotting. Refer to a standard protocol for your specific technique to determine if this is necessary.

You will find additional information below 'RECOMMENDED TRANSFER CONDITIONS & TRANSFER BUFFER' and 'TECHNICAL SUPPORT & ORDERING INFORMATION'.

Assembling the gel sandwich

The models SEDEC S and SEDEC M Semidry Electro Blotters are configured with the anode on the bottom and the cathode on the top. When assembling the gel sandwich, be sure to place the gel on top of the membrane, as negatively charged molecules will transfer in downward direction.

1. Stack three pieces of cut, presoaked blotting papers (3 mm) in the center of the base of the blotting system without any air bubbles in between.
2. Put a prepared blotting membrane of same size on top of the blotting paper stack.
3. Place the gel on top of the membrane. Remove any air bubbles between gel and membrane but avoid moving the gel once it got in contact with the membrane.
4. A pipette or test tube may be used to roll bubbles out of layers.
5. Finally stack another three pieces of presoaked blotting papers on top.
6. Wipe away any drips on the bottom plate prior to closing the lid - these may cause a short circuit allowing the current to bypass the stack and will cause poor transfer results. Sometimes it might even damage the electrode plates.
7. After the gel sandwich is assembled, the safety lid containing the cathode plate can get closed.
8. Fix the lid using the screwing knobs. The weight of the lid is enough to provide good contact and therefore excessive tightening is NOT required. If the lid is over tightened this may damage the gel, and force buffer out of the filter pads. Once the lid has been placed on top of the transfer sandwich it should not be moved. Any movement could create a smeary transfer, because transfer begins as soon as the membrane is in contact with the gel.
9. Please connect the banana plugs of the blotter to an appropriate power supply using the pair of power cords delivered. The power cords are color coded for the proper orientation of current. Start the transfer by defining the operating conditions (see 'RECOMMENDED TRANSFER CONDITIONS & TRANSFER BUFFERS').

Transfer of more than one gel

Up to four mini gels may be placed side-by-side for simultaneous transfer in the SEDEC M. If transferring more than one gel per apparatus, make sure that the gel sandwiches are the same thickness. Otherwise, the smaller of the two gel sandwiches may not make good contact with the top plate, and transfer will not occur.

Transfer buffers and transfer times

Buffers for semidry electric blotting must be low in ionic strength to avoid overheating. See 'Summary of appropriate transfer conditions' for some recommended buffers and running conditions. PEQLAB recommends running blots at constant current settings. Transfer times will vary, depending on the molecule's size, and percentage of the gel and to some extent, the transfer buffer. In general, small molecules transfer faster than larger ones. If transfer times are too long, small proteins (<15 kDA) might even pass the membrane if the pore size of the membrane is too large. A study by Tovey & Baldo (1987) showed that protein transfer was generally complete in 1-1/2 to 2 hours. Beyond that point, the buffer becomes depleted, so transferring for additional time will not improve efficiency. For an initial definition of transfer settings see 'RECOMMENDED TRANSFER CONDITIONS & TRANSFER BUFFER' also.

Current settings

Semi-dry blots should be performed at low voltage (around 10 V), preferential at constant current settings in order to avoid overheating and fluctuating power readings. For calculating the correct current setting, first determine the area of the gel(s), and then multiply that by the appropriate value for milliamps. In electrophoretic blotting, calculations are made in terms of current density. Proteins are blotted in a range of 0.8-3.0 mA/cm² and nucleic acids in the range of 0.5-3.0 mA/cm². When transferring a sequencing-sized gel, a setting of about 0.8 mA/cm² is appropriate. For example, using one 10 x 10 cm mini gel, the gel area is 10 x 10 = 100 cm². If you choose a current setting of 2 mA per cm², you would set constant current at 100 cm² x 2 = 200 mA. If you transfer more than one gel, add together the areas of the gels.

Read your power supply's instructions to assure that the power supply will work at a voltage lower than 10 V. These voltages often occur in semidry blotting. Contact the manufacturer regarding the unit's performance under high current, low voltage conditions if you have any questions. You will find a list of PEQLAB's Power Supplies below 'TECHNICAL SUPPORT & ORDERING INFORMATION'.

Factors that affect transfer efficiency

While general conditions can be described which will result in successful transfer of most molecules, it should be noted that optimal transfer conditions will vary based on the characteristics of the molecule you are working with. Some factors that affect transfer rate and efficiency include molecule size, charge, gel thickness and percentage, and hydrophobicity. The reference list at the end of this booklet provides useful information on how to choose optimal transfer conditions for a specific molecule.

If transfer conditions should be optimized, it is useful to stain the gel as well as the membrane using a general staining method (e. g. Coomassie blue or silver staining of protein gels and Ponceau red for proteins on membranes) and to compare the results with an identical gel which has not been blotted.

Cleaning

The interior of the basis and the lid of the blotter should get cleaned carefully after each use in order to remove any precipitates. Please use paper towels soaked with distilled water for that purpose. Each part should air dry completely before storage.

Do not use ethanol or other organic solvents to clean acrylic products, because organic solvents cause acrylic to 'craze' or crack!

RECOMMENDED TRANSFER CONDITIONS & TRANSFER BUFFERS

Summary of appropriate transfer conditions

	Proteins from polyacrylamide gels	DNA/RNA from agarose gels
Membrane	Nitrocellulose or PVDF (0.45 μm or 0.2 μm)	Nylon
Transfer buffer	3 Buffer System or Towbin Buffer	0.5x- to 1x TBE or 1x TAE or NAQ
Electrical conditions	constant current 0.8 to 3 mA per cm^2 gel size max. 10 to 14 Volt	constant current 0.5 to 3 mA per cm^2 gel size max. 10 to 14 Volt
Transfer time	30 minutes to 2 hours	30 minutes to 2 hours

Transfer buffers

3 Buffer System (Kyhse-Anderson, 1984)

Using this system the transfer will involve an isotachophoretic process, where the proteins are mobilized between a leading ion and a trailing ion. Anode buffer II neutralizes excess protons generated on the surface of the anode plate. Anode buffer I contains Tris at the same pH as anode buffer II, but at a reduced concentration of 25 mM. The cathode buffer contains 6-aminocaproic acid, which serves as the trailing ion during transfer and is depleted from the cathode buffer as it migrates through the gel toward the anode.

Blot-assembly:

1. stack 3 blotting papers soaked in anode buffer II inside the center of the blotter's base
2. put 3 blotting papers soaked in anode buffer I on top of the stack
3. put the blotting membrane on top, equilibrated in anode buffer I
4. place the gel on top, shortly (1 min) incubated in cathode buffer
5. finally, stack 3 blotting papers soaked in cathode buffer on top

Cathode buffer:

25 mM Tris-HCl, 40 mM 6-aminocaproic acid, 20% (v/v) Methanol, pH 9.4

Anode buffer I:

25 mM Tris-HCl, 20% (v/v) Methanol, pH 10.4

Anode buffer II:

300 mM Tris-HCl, 20% (v/v) Methanol, pH 10.4

Towbin Buffer (Towbin *et al.*, 1979)

The most commonly used buffer for protein blotting from polyacrylamide gels is Towbin buffer. However for difficult applications (e. g. transfer of large proteins, extended transfer times) the 3 Buffer System described above will be superior.

1x Towbin buffer:

25 mM Tris-Base, 192 mM Glycine, 20% (v/v) Methanol, pH 8.3

NAQ Northern Transfer Buffer (Trnovsky, 1992)

For transfer of RNA from agarose gels. With its high buffering capacity and low ionic strength, this buffer is more efficient than TAE, TBE or MOPS.

50x stock solution:

- 0.2 M Morpholinopropanesulfonic acid (MOPS)
- 50 mM Sodium acetate
- 5 mM EDTA
- pH 7.0

NAQ Southern Transfer Buffer

For transferring DNA from agarose gels.

50x stock solution

- 1M Ethanolamine-glycine buffer, pH 11

NAQ Transfer Buffer

For transferring nucleic acids from agarose gels.

10x stock solution:

- 0.8 M Tris-Base
- 1.18 M Boric acid
- 24 mM EDTA pH 8.3

TAE (Tris-Acetate-EDTA) Buffer

For transferring nucleic acids from agarose gels.

1x working solution:

- 40 mM Tris-Acetate, 1 mM EDTA

50x stock solution:

- 242 g Tris-Base
- 57.1 ml Glacial acetic acid
- 100 ml 0.5 M EDTA (pH 8.0)
- Adjust volume to 1L using distilled H₂O.

TBE (Tris-Borate-EDTA) Buffer:

For transferring nucleic acids from agarose gels.

0.5 x working solution:

- 45 mM Tris-Borate, 1 mM EDTA

5x stock solution

- 54 g Tris-Base
- 27.5 g Boric acid
- 20 ml 0.5 M EDTA (pH 8.0)
- ad 1 l using H₂O

TROUBLESHOOTING

Problem	Cause	Solution
Transfer efficiency is poor	Current is too low	The semidry transfer should be performed at constant current. Current density should be between 0.5 and 3 mA/cm ² of stack surface area.
	Power supply is inappropriate for semidry transfer	Many power supplies will shut off or blow a fuse when run at the conditions required for semidry transfer. Semidry transfer requires low voltage (often less than 10 V) and high current. Check with the manufacturer of the power supply to determine whether it is appropriate for semidry transfer. Appropriate Power Supplies from PEQLAB are listed below 'TECHNICAL SUPPORT & ORDERING INFORMATION'.
	Transfer time too short	Increase transfer time.
	Transfer sandwich was assembled in the wrong order	The Sedec™ Semi-dry blotters are configured with the anode on the bottom, and cathode on top. This means a downward transfer is being performed. Follow the instructions carefully when assembling the transfer sandwich.
	The pH of the transfer buffer is too close to the isoelectric point of the protein	Try a more acidic or basic transfer buffer.
	Too much methanol in the transfer buffer	Reducing methanol can help elute proteins from the gel, but can reduce binding to nitrocellulose membranes.
	High percentage gels restrict transfer	Higher percentage acrylamide or cross linker can restrict elution of proteins. Use the lowest percentage acrylamide possible to separate your proteins.
	Puddles of buffer were present on the anode allowing the current to bypass the stack	Always clean up the lower plate before closing the lid of the transfer apparatus. Do not squeeze the stack excessively as this also creates puddles that the current can pass through.
	The filter paper was too dry	Filter paper should be saturated with transfer buffer before adding them to the sandwich.

Problem	Cause	Solution
Smear or swirled transfer and missing bands	Air spaces are interfering with contact between the gel and the membrane	Roll a test tube or pipette over the gel (make sure it is clean) before putting the rest of the filter paper on the sandwich. Transfer will not occur where the gel is not in contact with the membrane.
	Electrophoretic conditions were incorrect or not ideal	Running conditions, sample preparation, percentage acrylamide, and many other variables can affect the migration and resolution of proteins. Please review your electrophoresis conditions.
Brown coloration of membrane or cracking of gel after transfer	Transferring at too high a current/temperature	Running at constant voltage can cause power fluctuations that will cause overheating. A buffer that has not been made correctly or that has too high in ionic strength can also burn a gel by overheating. A cracked and dry gel often is an indicator of overheating.
	Membrane was not thoroughly wetted	Always pre-wet the membrane according to the manufacturer's instructions. White spots indicate dry areas of the membrane.
Nitrocellulose membranes		
Insufficient binding of proteins to the membrane	Over-transfer through the membrane	Use 0.2 µm pore size nitrocellulose instead of 0.45 µm, or use PVDF with a higher binding capacity.
	Not enough methanol in transfer buffer	Nitrocellulose binds proteins best when 20 % methanol is used in the transfer buffer. This is especially important for small proteins (<20 kDa).
	SDS is preventing binding	Eliminate SDS in the transfer buffer.
PVDF-Membranes		
Smear or swirled transfer and missing bands	Membrane was dried out before it was added to the transfer sandwich	Membrane should be completely gray and slightly translucent when added to the sandwich. If it has dried out, rewet in methanol and equilibrate in transfer buffer.
	Alcohol was not used to pre-wet the membrane	PVDF is hydrophobic and requires a short soak in methanol prior to transfer.

TECHNICAL SUPPORT AND ORDERING INFORMATION

For technical questions and more detailed information on PEQLAB's products please visit www.peqlab.com to find the respective contact person.

PerfectBlue™ Semi-Dry Electro Blotter

Item	Description	Cat. No.
Sedec™ S	complete system for gels 10 x 10 cm	52-1010
Sedec™ M	complete system for gels 20 x 20 cm	52-2020

Power Supplies

Especially models EV261, EV202 und EV231 are recommended for electro blotting. Do not hesitate to contact us for advice on which Power Supply is most suitable for your application.

Item	Ports	max. Voltage (V)	max. Current (mA)	Power (W)	Cat. No.
EV222	3	200	200	20	55-EV222 ¹⁾
E300	4	300	500	90	55-E300-230V ²⁾
EV243	3	400	300	50	55-EV243 ¹⁾
EV231	4	300	1000	150	55-EV231 ¹⁾
EV265	4	600	500	150	55-EV265 ¹⁾
E250	4	250	3000	300	55-E250-230V ²⁾
EV202	4	300	2000	300	55-EV202 ¹⁾
EV261	4	600	1000	300	55-EV261 ¹⁾
EV215	4	1200	500	300	55-EV215 ¹⁾
EV232	4	3000	150	150	55-EV232 ¹⁾
EV233	4	3000	300	300	55-EV233 ¹⁾
EV262	4	6000	150	300	55-EV262 ¹⁾

¹⁾ Not available for costumers in the US.

²⁾ For a 110 V US version please replace '230V' with '110V' in the ordering number.

Blotting membranes¹⁾

Item	Pore size	Amount	Cat. No.
Nitrocellulose membrane	0.45 µm	0.30 x 3.0 m	39-1010
Nylon membrane	0.45 µm	0.30 x 3.0 m	39-2010
PVDF membrane	0.45 µm	0.30 x 3.0 m	39-3010
PVDF membrane	0.20 µm	0.30 x 3.0 m	39-4010

¹⁾ Not available for customers in the US.

LITERATURE

Semi-Dry blotting

Bjerrum, O.J. and Schafer-Nielsen, C. (1986) in: Dunn, J.J. (ed.) *Electrophoresis '86* VCH Weinheim, pp. 315-327. These authors compare results using different transfer buffers (Towbin buffer vs. the three buffer system).

Khyse-Anderson, J. (1984) Electroblotting of multiple gels. A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide gels to nitrocellulose. *J. Biochem. Biophys. Methods* 10: 203-209. This paper describes a the semidry blotter with a 3 buffer system which is effective for transfer of proteins.

Electroblotting of proteins

Castora, Frank J. (1989) Western Blotting of Proteins, *Clinical Biotechnology* 1: 43-49. This review article on Western Blotting gives a good overview of factors such as transfer buffers, types of membranes, and post-membrane stains. Although written for standard tank blotting, much of this is applicable also to semidry blotting.

Dunbar, B.S., Ed. (1994) *Protein Blotting: A Practical Approach*. IRL Press at Oxford University Press, Oxford, England. A great guide to blotting techniques, including visualization, immunological techniques, and sequence analysis.

Eckerskorn, Christoph and Lottspeich, Friedrich (1993) Structural characterization of blotting membranes and the influence of membrane parameters for electroblotting and subsequent amino acid sequence analysis of proteins, *Electrophoresis* 14: 831-838. A useful reference if you plan to do protein sequencing of samples transferred samples.

LeGendre, Nancy (1990) Immobilon-P Transfer Membrane: Applications and Utility in Protein Biochemical Analysis", *BioTechniques suppl to vol 9*: 788-805. This references deals specifically with transfer conditions using Immobilon-P type membranes.

Tovey, E.R. and B.A. Baldo (1987) Comparison of semidry and conventional tank-buffer electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes. *Electrophoresis* 8: 384-387. This paper discusses quantitative yields of proteins of different molecular weights using different transfer conditions.

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

Electroblotting of nucleic acids

Trnovsky, Jan (1992) Semidry Electroblotting of DNA and RNA from Agarose and Polyacrylamide Gels, *BioTechniques* 13: 800-804.

NOTES



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AT
UK
USA

PEQLAB Biotechnologie GmbH, 91052 Erlangen, Freecall (D): 0800 100 20 16, info@peqlab.de, www.peqlab.de
PEQLAB Biotechnologie GmbH, 6404 Polling, Tel: +43 (0) 5238 84 169, info@peqlab.at, www.peqlab.at
PEQLAB Ltd., Fareham PO15 5TT, Freecall (UK): 0808 202 1302, info@peqlab.co.uk, www.peqlab.co.uk
PEQLAB LLC, Wilmington, DE 19810, Toll-Free (US): 877 737 5220, info@peqlab.us, www.peqlab.us

Creating the future together.