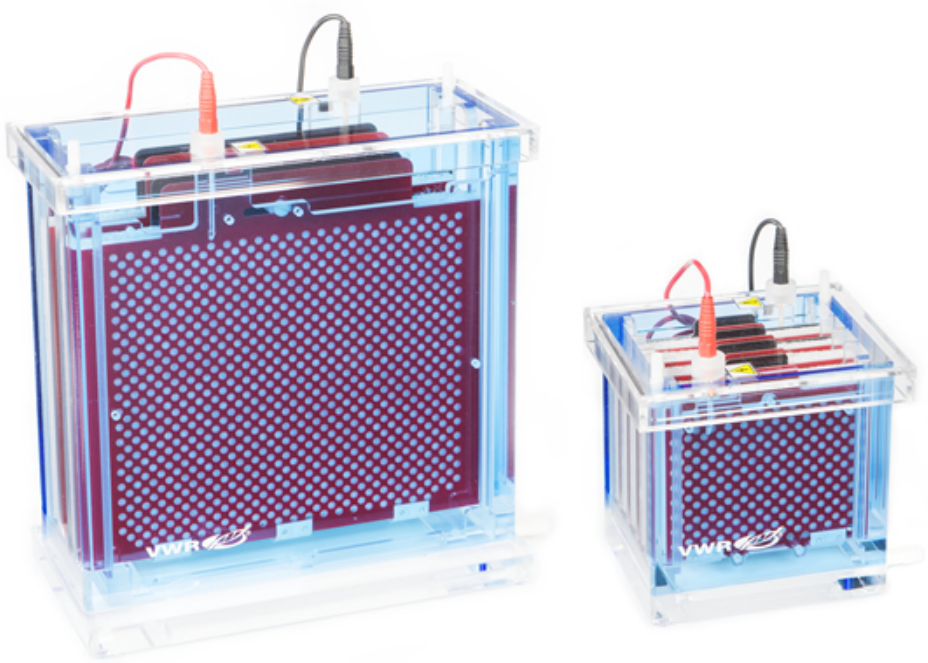
VWR® PerfectBlue™ Tank-Electroblotter Web S & Web M

Instruction Manual



European Catalogue Numbers:

Web S	**700-1228**
Web M	**700-1230**

Version: 1

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CE

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Legal Address of Manufacturer

VWR International bvba

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Country of Origin

United States

Warranty

VWR warrants that this product will be free from defects in material and workmanship for a period of three (3) years from date of delivery. If a defect is present, VWR will, at its option and cost, repair, replace, or refund the purchase price of this product to the customer, provided it is returned during the warranty period. This warranty does not apply if the product has been damaged by accident, abuse, misuse, or misapplication, or from ordinary wear and tear. If the required maintenance and inspection services are not performed according to the manuals and any local regulations, such warranty turns invalid, except to the extent, the defect of the product is not due to such non performance.

Items being returned must be insured by the customer against possible damage or loss. This warranty shall be limited to the aforementioned remedies. IT IS EXPRESSLY AGREED THAT THIS WARRANTY WILL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND IN LIEU OF THE WARRANTY OF MERCHANTABILITY.

Packaging List

The following items are included in shipment for the models Web™ S and Web™ M:

- one buffer tank with plate electrodes and 2 hoses barbs for external cooling
- one safety lid with attached power cords
- Web S: 4 color-coded cassettes including 2 fiber pads each
- Web M: 2 color-coded cassettes including 2 fiber pads each
- one instruction manual

Safety Precautions

To avoid electrical shock:

- 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.

Intended Use

Use this product only for its intended purpose as described in this manual. Do not use this product if the power leads are damaged or if any of its surfaces are cracked.

For research use only. Not intended for use in diagnostic or therapeutic applications.

System Overview

The PerfectBlue™ Tank electro blotting systems Web S and M are designed to rapidly and quantitatively transfer nucleic acid or proteins from up to four polyacrylamide gels at once to nitrocellulose, nylon or PVDF

membranes. The color-coded cassettes allow for easy assembly of blotting sandwich and error-free transfer. The robust acrylic construction of the buffer tank and the safety lid withstands daily usage without breakage, warping or leakage.

Gold-plated banana plugs together with solid plate electrodes made of platinum-plated titanium (anode) or stainless steel (cathode) are assuring highest electric conductivity and an exceptional homogeneity of the electrical field. As a result transfers of outstanding quality will be achieved. For effective heat removal a water circulation made of alumina ceramic is integrated in the base of the buffer tank allowing for shortest transfer times using high currents.

Buffer chambers of PerfectBlue™ wet blotting systems Web S or Web M offer space for up to 4 or 2 blotting cassettes, respectively, and therefore enable the user to transfer samples from several gels at once. In addition there is space for a magnetic stir bar which should be used to assure uniformity of temperature and ions.

Technical Properties

PerfectBlue™	Cat. No.	Transfer area	Buffer volume	Current	typ. transfer time
Web S	700-1228	8.5 x 9.5 cm	approx. 1300 ml*	200-500 mA	120-360 min
Web M	700-1230	18 x 20 cm	approx. 4500 ml*	1000-1500 mA	120-360 min

General Instructions

Materials Required

Before getting started, the required transfer buffer, blotting papers and blotting membranes should be prepared. Blotting papers membranes will be cut according to the gel size and moistened in transfer buffer. In general 2 layers of blotting paper (3 mm) each will be placed above and below the respective membrane or gel. The number of blotting papers can be increased if the fiber pads became thinner from repeated usage, in order to assure tight fitting of the blotting sandwich inside the blotting cassettes.

The required amount of transfer buffer (see 'Technical properties') should be cooled and degassed in order to minimize the formation of air bubbles during transfer. The way of preparing the blotting membrane is dependent on its nature. Please follow the manufacturer's instructions. In some cases an equilibration of the gel in transfer buffer might be necessary. Please follow the instructions of your standard protocol. You will find additional information below 'Recommended Transfer Conditions and Transfer Buffer' and 'Technical Support and Ordering Information'.

Assembling the Gel Sandwich

For tank electro blotting systems Web™ S and M electrode plates are located inside the side walls of the buffer tank. Electrode plugs and blotting cassettes, in which the blotting sandwich will get mounted, are color-coded to assure proper orientation. For gel sandwich assembly, mounting of blotting cassettes inside the buffer tank, and connection to power supply, please take care that the membrane is located at the side of the gel which is close to the anode (positive electrode , red-colored) if your target molecules are negatively charged.

1. After electrophoresis, remove the gel assembly from the unit and remove the spacers
2. Open the gel cassette by gently rocking a spatula between the plates, forcing separation of the plate from the gel – do not insert spatula near the notch of the glass. The gel will normally remain affixed to the bottom plate. Remove the top (notched) plate by slowly lifting it from the side with the inserted spatula and gradually increasing the angle until the plate is completely separated from the gel.
3. If the gel should stick in an isolated spot, a stream of water from a squirt bottle at the spot will aid separation.
4. Remove the gel from the bottom plate. Tip the plate up side down, starting at one edge allow the gel to roll off into the transfer buffer. Alternatively, place the plate with the gel attached, into transfer buffer.
5. Pre-equilibrate the gel in cool transfer buffer for 15 minutes with gentle agitation to remove SDS and salts. This serves to prevent the gel changing size during transfer and to reduce heating effects. If the gel is on the plate, it will become loose during this step.
6. Wearing gloves, cut the membrane to the size of the gel and blotting paper.
7. Mark the membrane to indicate the side that the samples will be on. This is important to indicate sample orientation in the event that any successive probe of the membrane is negative. Either clipping a corner of the membrane or using a ballpoint pen can do this.

8. Wet the membrane according to the manufacturer's recommendations, followed by equilibration in transfer buffer for 15 minutes.
9. Cut four pieces of laboratory grade blotting paper to a size 5mm larger than the gel to be blotted. Soak them in transfer buffer.
10. Soak fiber 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.
11. Building the gel sandwich is best done in a dish or shallow plastic box. Place the cassette with the black side down.
12. Lay a fiber pad on the black half of the cassette, followed by the soaked filter paper. Both should be soaking wet with transfer buffer. The tray may become filled with buffer as you build the blotting sandwich.
13. Add a few ml of buffer to the filter papers and gently layer the gel. Beginning at one end of the gel, align the filter papers with the gel edge and slowly lower the other end, driving out any bubbles. Use a clean glass rod wetted in transfer buffer to roll out any trapped bubbles.
14. Carefully align and overlay the transfer membrane onto the gel in one smooth action. Work from the center and let the ends progressively roll down. Do not attempt to re-position the membrane as some transfer may occur on contact. Again, use a clean glass rod wetted in transfer buffer to roll out any trapped bubbles.
15. Add another two pieces of wetted filter paper and complete the sandwich with a fiber pad.

Setting Up the Tank

1. Cool and degas the appropriate volume of transfer buffer (see 'Technical properties').
2. Half fill the tank with transfer buffer.
3. Connect the cooling water supply to the tank base hose connectors. Use hose clips to secure the tubing.
4. Add a stir bar to the tank to maintain uniform temperature and conductivity during electrophoretic transfer
5. Insert the cassette into one of the tank slots with the transfer membrane on the anode (positive/red) side.
6. Add transfer buffer until the top of electrode plates are just covered.
7. Place the safety lid on the unit and connect the attached power cords to an appropriate power supply. Start the transfer by defining the operating conditions (see 'Recommended Transfer Conditions and Transfer Buffers').

Transfer of More Than One Gel

Using the PerfectBlue™ electro blotting system Web S or Web M up to four or two blotting cassettes can get used at once, respectively. However, it has to be noticed that transfer efficiencies might decrease if several blotting cassettes are inserted. In this case, transfer times have to be increased in order to achieve the same transfer efficiencies as if blotting a single gel sandwich.

Transfer Times and Conditions

Transfer times must be determined experimentally. There is no formula for determining transfer time since there are too many variables involved to give specific transfer conditions. Initially, choose a buffer system 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 settings, monitoring the temperature as the run proceeds. If the temperature is controlled and transfer is successful you can try higher settings and decreased transfer times for subsequent runs. The following formula is describing the relation between current and transfer time:

Current (mA) x Transfer time (h) = Transfer efficiency (mAh)

If transfer conditions should get 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.

For an initial definition of transfer settings see 'Recommended Transfer Conditions and Transfer Buffer' also.

Power Supplies

Blotting requires a power supply that operates at a fairly high current setting and low voltage (often below 10 V). If the power supply used is not appropriate it may blow a fuse, shut itself off, display a no load or short load message or even have a short circuit. Please contact your Power Supply's manufacturer or if it is unclear if your Power Supply matches the requirements.

Cleaning

The buffer tank, plate electrodes, blotting cassettes and fibre pads should be rinsed under warm running water after each use. Use a mild detergent to get rid of any debris. Rinse with distilled water afterwards. 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 and Transfer Buffer

Summary of Appropriate Transfer Conditions

	PerfectBlue™ Web S		PerfectBlue™ Web M	
	over night	1 h	over night	1 h
Towbin Buffer	25 to 40 V 40 to 80 mA	50 to 100 V 200 to 400 mA	25 to 40 V 80 to 160 mA	50 to 60 V 200 to 1250 mA
Bjerrum Buffer	25 to 40 V 40 to 80 mA	50 to 100 V 200 to 400 mA	25 to 40 V 80 to 160 mA	50 to 60 V 200 to 1250 mA
Dunn Buffer	10 V 40 to 80 mA	40 to 80 V 200 to 500 mA	10 V 80 to 160 mA	50 to 60 V 200 to 1300 mA

Please determine optimal conditions experimentally for your specific application starting from these recommendations (see 'Transfer Times and Conditions').

Transfer Buffers

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

The most commonly used buffer for protein blotting from polyacrylamide gels is Towbin buffer. In order to increase transfer efficiency of proteins from the gel 0.05 to 0.1% SDS might be added. However, this might lower binding capacity of the membrane. The buffer should be degassed prior to the addition of SDS and cooled to 4 ˚C.

25 mM Tris-Base, 192 mM Glycine, 5 - 20 % (v/v) Methanol, pH 8.3

Bjerrum-Puffer (Bjerrum & Schafer-Nielsen, 1986)

48 mM Tris-Base, 39 mM Glycine, 5 - 20 % Methanol, pH 9.2

Dunn-Puffer (Dunn, 1986)

10 mM $NaHCO_3$, 3 mM $NaCO_3$, 20 % MeOH, pH 9.9

Blotting Membranes

Nitrocellulose and PVDF (Polyvinylidene difloride) can be used for proteins. Charged Nylon Membranes can be used for nucleic acids. The choice depends upon the user’s preference and sometimes the detection method to be used afterwards. You will find information upon VWR's blotting membranes below 'Technical Support and Ordering Information'.

Troubleshooting

Problem	Cause	Solution
Transfer efficiency is poor	Current too low	Increase the current in the range given below 'Summary of appropriate transfer conditions'.
	Transfer time too short	Increase transfer time
	Power supply is inappropriate for transfer	Many power supplies will shut off or blow a fuse when run at the conditions required for electro blotting like low voltage (often less than 10 V) and high current. Use the instruction manual of your power supply to determine whether it is appropriate or not. Appropriate Power Supplies from VWR are listed below 'Technical Support and Ordering Information'.
	Transfer sandwich was assembled in the wrong order or mounted wrongly oriented inside the buffer tank	Placing the membrane on the wrong side of the gel is the most common reason for 'empty' membranes. Usually the membrane should be closest to the anode. 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.
Smeared or swirled transfer and missing bands	Air bubbles inside the fiber pads or between the different layers of the blotting sandwich	Air bubbles will disrupt the electrical field and interfere with transfer. Avoid the presence of air bubbles inside the blotting sandwich.
	Gel and membrane moved relative to each other prior to or during transfer	Take care that gel and membrane do not move after getting in contact to each other. The number of blotting papers can be increased if the fiber pads became thinner from repeated usage, in order to assure tight fitting of the blotting sandwich inside the blotting cassettes.
	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 chosen for the experiment prior to blotting.

Problem	Cause	Solution
Brown coloration of membrane or cracking of gel after transfer	Transferring at too high a current/temperature	Maybe the current was too high. Please refer to the running conditions given below 'Summary of appropriate transfer conditions'. Use the cooling option offered by the PerfectBlue™ Wet Blotting systems. Check for correct buffer composition.
	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		
Smeared 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 prewet 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 VWR's products please contact your local VWR office or visit the VWR website at **vwr.com**.

Visit the VWR website at **vwr.com** for:

- Complete technical service contact information
- Access to the VWR Online Catalogue, and information about accessories and related products
- Additional product information and special offers

PerfectBlue™ Wet Blotting Systems

Article	Description	Bestell-Nr.
PerfectBlue™ Web S	complete system for gels up to 8.5 x 9.5 cm	700-1228
PerfectBlue™ Web M	complete system for gels up to 18 x 20 cm	700-1230
Blotting cassettes	for Web™ S, 1 cassette incl. 2 fiber pads	700-1273
	for Web™ M, 1 cassette incl. 2 fiber pads	700-1252
Fiber pads	for Web™ S, 4 pads 10 x 12.5 cm	700-1274
	for Web™ M, 4 pads 18 x 24.5 cm	700-1256

Power Supplies

Do not hesitate to contact us for advice on which Power Supply is most suitable for your application.

Blotting Membranes

Item	Pore size	Amount	Cat. No.
Nitrocellulose membrane	0.20 µm	0.30 x 3.0 m	732-3197
Nylon membrane	0.45 µm	0.30 x 3.0 m	732-3198
PVDF membrane	0.20 µm	0.30 x 3.0 m	732-3199
PVDF membrane	0.20 µm	0.26 x 3.3 m	732-3200

Literature

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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).

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SAMBROOK, J., FRITSCH, E.F. MANIATIS, T. (1989) Molecular Cloning. A Laboratory Manual 2nd Edition. 18.47-18.61. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

TOWBIN, J., STAEHELIN, T., and GORDON, J. (1979) Electrophoresis transfer of proteins from polyacrylamide gel to nitrocellulose sheets. Procedure and some applications, Proc. Natl., Acad. Sci. USA, 76, 4350-4354.

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