Instructions for use

Semi dry blotter ROTIPHORESE® PROfessional

MAXI (Art. No. KK59.1), MINI (Art. No. KK58.1)

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.

The Roth Semi Dry 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.
DO NOT attempt to remove the outer casing or make repairs to our electrical range of products, should any unit fail.

WARRANTY

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. No liability is accepted for loss or damage arising from the incorrect use of this unit.

DESCRIPTION:

The Roth Semi Dry Blotters may be used for blotting of DNA, RNA and proteins with transfer times of typically 30 to 60 minutes, very thin gels may also be blotted in 15 minutes only. These units may be used with gels thicknesses from 0.25 mm up to 10 mm using all standard blotting buffers – continuous as well as discontinuous buffer systems – in Western-, Southern- and Northern blotting procedures. The electrodes, comprising platinum coated anode and stainless steel cathode, will exhibit practically no corrosion. Uniform heat dispersion across the blot sandwich ensures stable transfer times and no heat induced sample loss or transfer distortions.

TECHNICAL DATA:

- Rugged acrylic construction, all details chemically bonded
- Gold plated electrical connectors, corrosion-free and rated safe up to 1,000 volts.
- Platinised Titanium positive electrode (anode)
- Stainless Steel negative electrode (cathode)
- Maximal blotting area: 10 x 10 cm (MINI) / 20 x 20 cm (MAXI)
- Unit size (l x w x h): 16 x 16 x 7 cm (MINI) / 26 x 26 x 7 cm (MAXI)

PACKING LIST:

1 Semi Dry Blotter with lid.
2 Leads with connectors
1 Instruction manual

ENVIRONMENTAL CONDITIONS:

- This apparatus is intended for indoor use only.
- This apparatus can be operated safely at an altitude of 2,000 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”.

QUALITY CHECK

All Roth products are supplied having passed rigorous quality control procedures. If however, you have a query of any nature, please call (+49) 721 5606-0.

OPERATING INSTRUCTION:

A Safety Precautions

- READ instructions before using the apparatus.
- Always isolate the 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 current or voltage.
- DO NOT operate the units in metal trays.
- DO NOT move the unit when it is running.

PLEASE NOTE:

Cable male connectors of the Semi Dry Blotters are equipped with fixed sheath. In case these connectors do not fit the female connector of your Power Supply we recommend use of the adaptor pair Art. No. K295.1

OPERATIONAL (MIN):

Maximum operating current: 550 mA (MINI) / 1200 mA (MAXI)
Maximum operating voltage: 75 Volts (MINI, MAXI)

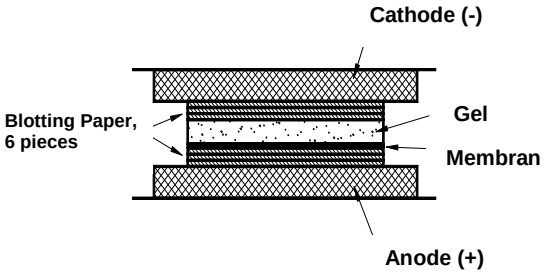
B General Care and Maintenance

- Before removal of the safety lid, make sure that the leads are disconnected and lift the lid vertically with your fingers.
- The unit 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 plate electrodes and vigorous cleaning is not necessary or advised.
- Air drying is preferably before use.
- Units are best cleaned using warm water and a mild detergent (e.g. soap). Water at temperatures above 60 °C can cause damage to the unit and components. Compatible detergents include dishwashing 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.
- Ensure that the connectors are clean and dry before usage or storage.

In most cases, **RNAse Decontamination** is not necessary at all. If, however, RNAse decontamination shall be performed nevertheless, this may be done according to the following protocol:
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 pyro carbonate) treated distilled water,
Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using.
ROTI®Nucleic Acid free (Art. No. HP69) and RNAse AWAY(TM) (Art. No. A998) may also be used. Please consult the instructions for use with acrylic gel tanks.

C Setting up the Blot Sandwich

Please see below for details on blotting buffers. Given numbers of sheets of blotting paper are meant for paper of thickness approx. 0.35 mm. Adjust amounts accordingly for thicker grade filter paper.



1. Place the gel in cathode buffer (i.e. ROTI®Blot K) and agitate gently.
2. Cut 12pieces of blotting paper (0.35 mm thick) and a piece of blotting membrane to the exact size of the gel to be blotted. **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 as well as subsequent false signals.
3. Depending on the type of membrane used, the membrane may need equilibrating in buffer prior to blotting. In case you are using PVDF-membrane, activate the membrane in 100 % Methanol. Nitrocellulose should be equilibrated in anode buffer. Please also consult the membrane manufacturer's guidelines for wetting the membrane prior to blotting.
4. Ensure that excess liquid drains from the membrane.
5. Take 6 pieces of the cut blotting paper and soak in the cathode buffer (ROTI®Blot K).
6. Take 6 pieces of the cut blotting paper and soak in the anode buffer (ROTI®Blot A).
   *Make sure that there is **at least** 2 mm blotting paper at each side of the sandwich. Too little paper limits*

the amount of buffer ions available for the blot, resulting in lower transfer efficiency and, in the long run, leading to damage of the electrode surface.

7. Mark the gel side of the membrane and clip one corner of the gel for subsequent orientation. Take a note of these marks.
8. Remove the lid from the blotter and place six filter paper pads pre-soaked in anode buffer onto the base electrode plate (anode, see figure) ensuring that any excess liquid is wiped away.
9. Carefully place the membrane exactly 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, or be removed by rolling a pipette or the ROTILABO®-sealing roller over the surface of the membrane.
10. 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.
11. Place the remaining six filter paper pads on top of the membrane and gently smooth.
12. Again check whether all blotting papers, membrane and gel are placed straight and exactly on top of each other.
13. Again roll with a glass rod (e.g. pipette) or using the ROTILABO®-sealing roller over the stack with light pressure, in order to ensure removal of any residual air bubbles from the sandwich.
14. 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.

NOTE: If more than one gel is to be blotted under the same conditions, simply stack them as previously described, with a piece of dialysis membrane presoaked in distilled water. This prevents small proteins from traveling through the blotting membrane and contaminating the one below.

D Running the Blot

1. 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.
2. 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 electrodes.
3. Set the blotter should at 2-5 mA per centimeter squared of gel, e.g. an 8 x 7 cm gel should run at approx. 110 mA. This results in voltages of approx. 10-50 V.
   Do not exceed 75 V or 550 mA (MINI) or 1200 mA (MAXI).
4. The actual run time will depend on the size of the molecules to be transferred. Large proteins and long nucleic acids will need 2 hours, small molecules less than 1 h. We recommend transfer time of 1 hour for the first blot performed with a special set of molecules.
   Note: The efficiency and quality of transfer depends on the type of buffer used, also the type of samples, and the parameters used for the run. Generally, better results can be obtained by reducing the power settings and increasing the blot time.

E Recommended Material

Accessories

ROTILABO®-sealing roller HE23.1

Power Supplies

Semi Dry Blotting generally requires high current settings >250 mA and the power supply should contain these capabilities. We recommend one of the following units:

Roth Power Supply BLOT (max. 300 V, 3000 mA, 300 W)	2909.1
Consort Power Supply EV3610 (max. 600 V, 1000 mA, 300 W)	2804.1
Consort Power Supply EV3020 (max. 300 V, 2000 mA, 300 W)	A543.1

Membranes

Membrane	Pore Size	Units	Amount	Size	Art. No.
ROTI®Fluoro PVDF	0.2 µm	Sheets	2	20 x 13 cm	2831.1
		Roll	1	330 x 26 cm	2803.1
ROTI®PVDF	0.45 µm	Roll	1	375 x 26,5 cm	T830.1
ROTI®NC	0.2 µm	Roll	1	30 x 300 cm	HP40.1
		Roll	1	20 x 300 cm	HP41.1
		Sheets	10	10,2 x 13,3 cm	HP42.1
		Sheets	10	20 x 20 cm	HP43.1

Blotting Paper

Thickness	Amount	Size	Art. No.
0.18 mm	100	10 x 13 cm	CL68.1
	100	20 x 20 cm	CL69.1
	100	46 x 57 cm	CL70.1
	100	58 x 60 cm	CL71.1
0.35 mm	100	10 x 13 cm	CL64.1
	100	20 x 20 cm	CL65.1
	100	46 x 57 cm	CL66.1
	100	58 x 60 cm	CL67.1
1.0 mm	25	15 x 15 cm	CL72.1
	25	20 x 20 cm	CL73.1
	25	58 x 60 cm	CL74.1
1.5 mm	25	58 x 60 cm	CL75.1

Buffers

General note: Transfer buffers must be made accurately using high grade reagents. pH and buffer quality will vary according to the purity of the reagents used.

Protein (Western) Blotting Buffers:

Do not use cathode buffers with pH values of under 8.3, since electrodes may take damage with frequent use. For semi dry blotting we recommend use of our ROTI®Blot buffer system, a discontinuous buffer system optimized for semi dry blotting including an anode buffer of pH 7.8±0.1 and a cathode buffer of pH 8.5±0.1: ROTI®Blot 1 (Art. No. L509.1).

Additionally, the following buffers may also be used (examples):
Buffer acc. to Tobwin et al. (1997): 25 mM Tris Base, 192 mM glycine, 20 % methanol, pH 8.3 (optional: 0.02 % SDS)
Buffer acc. to Bjerrum und Schaefer-Nielsen (1986): 48 mM Tris Base, 39 mM glycine, pH 9.2. Optional: max. 20 % methanol (recommended: addition of 10 % methanol to anode buffer, since methanol enhances binding of proteins to the membrane), optional: max. 0.1 % SDS (recommended: addition of 0.02 % SDS to cathode buffer, since SDS enhances movement of proteins in the gel and towards the membrane)
Triple buffer system:
Anode Buffer 1: 3 mM Tris Base, 20 % methanol, pH 10.4 (4 filter paper sheets)
Anode Buffer 2: 25 mM Tris Base, 20 % methanol pH 10.4 (2 filter paper sheets)
Cathode Buffer: 25 mM Tris Base, 40 mM caproic acid, 20 % methanol pH 9.4

DNA (Southern) Blotting Buffer:
1x (or 0.5x) TAE or 1x (or 0.5x) TBE

RNA (Northern) Blotting Buffer:
20 mM morpholinopropanesulfonic acid (MOPS), 5 mM sodium acetate, 1 mM EDTA, pH 7.0
(prepare 10x stock solution with DEPC treated water, adjust pH with NaOH. Sterilize by passing through a 0.45-µm filter. Store at RT protected from light. The buffer gets straw-coloured with time, discard if it gets darker.)

Reagents	**Art. No. ***
ROTI®Blot 1 for standard proteins	L509
Tris Base, Blotting-Grade	0188
Tris-HCl, p.a.	9090
Glycin, Blotting-Grade	0079
Methanol, Blotting-Grade	0082
SDS, Blotting-Grade	0183
ROTI®Stock 20 % SDS	1057
Caproic acid	8799
Morpholino propane sulfonic acid (MOPS)	6979
Sodium acetate, trihydrate, p.a.	6779
EDTA disodium salt dihydrate, p.a.	8043
ROTIPHORESE® 50x TAE Buffer	CL86
ROTIPHORESE® 10x TAE Buffer	T845
ROTIPHORESE® 10x TBE Buffer	3061

*** For package sizes and safety information please see our catalogue or online at www.carlroth.com**

TROUBLESHOOTING

Nucleic Acids	
Poor nucleic acid transfer	Transfer apparatus assembled incorrectly and proteins moving in the wrong direction ▪ Gel/membrane sandwich may be assembled in the wrong order. Check polarity.
DNA / RNA remains within gel	▪ Gel too hot and buffer too concentrated, resulting in excessive current and the gel starting to melt. Remake buffer to 0.5 X TBE, the concentration required for proper transfer ▪ Power conditions changed during transfer. Important to maintain constant current. If buffer less concentrated than 0.5 X, higher voltage is required to maintain recommended current, and vice versa. If voltage is too low, current will also fall below optimum setting, reducing migration. Increase voltage limit on power supply. ▪ Optimum transfer of plasmid, vector and PCR DNA achieved using settings recommended within the section: 'Running the Blot'
Poorly blotted or diffused transfer	▪ Poor contact between agarose and transfer membrane. Roll out gel with pipette before transfer to remove air and buffer bubbles. Repeat for blotting paper. ▪ Gel too thin, causing uneven electrical contact between the gel stack and electrodes. A 6-mm-thick gel and extra-thick blotting paper are recommended for full electrical contact. ▪ The gel may be too hot. Refer to DNA / RNA remains within the gel. ▪ Very small DNA fragments will diffuse during electrophoresis and blotting even if run in high percentage gels. Resolution is not always improved by running high percentage gels. ▪ Transfer membrane used might not properly bind DNA or RNA. Try a control membrane, a different lot or brand
Poor Detection Sensitivity	▪ Poor DNA probe labeling ▪ Insufficient signal hybridized to target DNA for detection. Labeled DNA probe not properly labeled. Check labeling controls to ensure that correct template DNA is being used and that reaction is working properly. ▪ Incomplete transfer of target DNA from gel to membrane. See **'DNA / RNA Remains within the Gel'**. Check agarose gel following transfer to determine whether transfer occurred or not. ▪ Specific activity of the probe may not be high enough for standard detection conditions. Determine specific activity and total cpm of probe added during hybridization. ▪ Hybridization conditions may be too stringent; alter to reduce stringency and improve efficiency of probe-template binding
High Background	▪ Increase hybridization stringency to reduce non-specific probe binding.

Protein	
Poor protein transfer	Transfer apparatus assembled incorrectly and proteins moving in the wrong direction • Gel/membrane sandwich may be assembled in the wrong order. Check polarity.
	Western detection system not working or not sensitive enough ▪ Include proper positive or negative control antigen. Consult kit manual. ▪ Use protein markers with coloured reference bands during PAGE. ▪ Stain gel with Coomassie, or stain membrane with Ponceau S.
	Transfer time too short – increase transfer time
	Power setting too low ▪ Check current at beginning of run. Current may be too low for a given voltage setting. Increase current if necessary but do NOT exceed 550 mA / 1200 mA. ▪ Buffer may be prepared improperly prepare new buffer and increase voltage
	Charge-to-mass ratio incorrect for native transfers. ▪ Proteins close to isoelectric point (pI). Change buffer pH so that it is at least 2 pH unit higher or lower than pI of protein of interest.
	Defective or inappropriate power supply used. ▪ Check fuse of power supply. Ensure max. current output of power supply is at least 2000 mA.
	Excessive methanol restricting transfer. ▪ Reduce methanol concentration to maximize protein transfer from gel, but without reducing concentration to the extent that it prevents binding to nitrocellulose. Alternatively reduce methanol concentration and switch to PVDF.
Protein precipitating in gel	▪ Use SDS in transfer buffer (SDS can increase transfer efficiency, but it can also reduce nitrocellulose binding affinity and affect protein-antibody reactivity). ▪ Remove alcohol from transfer buffer.
Swirls or missing bands; diffuse transfers	Poor gel-membrane contact. ▪ Air bubbles or excess buffer remain between membrane and gel. ▪ Carefully remove air bubbles between gel and membrane using a rolling pin ▪ Use more, or thicker, filter paper in gel-membrane sandwich
	Membrane not fully wet or has dried out ▪ White spots on nitrocellulose membrane indicate dry areas to which proteins will not bind. Ensure membrane is completely immersed in transfer buffer. ▪ If soaking does not occur immediately following immersion in transfer buffer, heat distilled water to just below boiling point and soak membrane until entirely wet. ▪ If using PVDF, immerse membrane in methanol before soaking in transfer buffer.
	Problem with gel electrophoresis. ▪ Poor gel polymerization, inappropriate running conditions, buffer contamination, excessive sample application all contribute to poor quality gels and transfers.
Poor binding to membrane - nitrocellulose	Excessive methanol restricting transfer. ▪ Ensure methanol concentration does not exceed 20 % (v/v).
	Proteins may be transferring through nitrocellulose. ▪ Use PVDF or smaller pore size (0.2 µm) nitrocellulose. ▪ Overlay an extra piece of nitrocellulose over membrane to determine if proteins are migrating through the membrane directly in contact with the gel.
	Proteins <15 kDa have reduced binding to 0.45 µm nitrocellulose or may be washed from membrane during assays. ▪ Use PVDF or nylon membrane, which have higher binding capacities.

	▪ Use Tween-20 detergent in the wash and antibody incubation steps. Reduce or eliminate the more stringent washing steps.
	SDS in transfer buffer reducing binding efficiency ▪ Reduce or eliminate SDS concentration
	Membrane incompletely wet ▪ White spots indicate dry areas where protein will not bind. ▪ If soaking does not occur immediately following immersion in transfer buffer, heat distilled water to just below boiling point and soak membrane until entirely wet.
Poor binding to membrane PVDF	Membrane is not completely wet ▪ Because of hydrophobicity of PVDF, the membrane must be soaked entirely in methanol before equilibration in aqueous buffer
	Proteins might be transferring through the membrane ▪ Decrease voltage if transferring under high intensity conditions ▪ Overlay an extra piece of PVDF over membrane to determine if proteins are migrating through the membrane directly in contact with the gel.
	Membrane might have dried during handling ▪ Fully wet membranes have a grey translucent appearance. White spots will form on the surface if the membrane has been allowed to dry. As proteins will not bind to dry spots, re-soak the membrane in methanol and re-equilibrate in transfer buffer
	SDS in transfer buffer reducing binding efficiency ▪ Reduce or eliminate SDS concentration
Power is too high	▪ Always check current at the start of the run, for the current might be too high for a given voltage setting. Improper buffer preparation can also result in high conductivity and excessive power generation. The current setting should not be allowed to exceed 2000 mA.
Immune specific detection and total protein detection	**Overall high background** ▪ Reduce antibody / protein sample concentration ▪ Blocking of unspecific antibody binding sites might not be sufficient (use BSA instead of milk powder and vice versa, higher concentration of blocking agent, longer incubation time) **Too low background/signal** ▪ Increase antibody concentration / protein sample concentration

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