user manual

ECL Semi-dry Blotters

TE 70 Semi-dry transfer unit
TE 77 Semi-dry transfer unit
TE 70 PWR Semi-dry transfer unit
TE 77 PWR Semi-dry transfer unit

um 28-4025-91/Rev. A0/09-05

Amersham Biosciences

Page finder

English

Important user information

Please read this entire manual to fully understand the safe and effective use of this product. Should you have any comments on this manual, we will be pleased to receive them via email at ts-usa@ge.com or at:

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Français

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Español

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Deutsch

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Italiano

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Waste Electrical and Electronic Equipment (WEEE) information

English

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of your equipment.

Français

Ce symbole indique que les déchets relatifs à l'équipement électrique et électronique ne doivent pas être jetés comme les ordures ménagères non-triées et doivent être collectés séparément. Contactez un représentant agréé du fabricant pour obtenir des informations sur la mise au rebut de votre équipement.

Deutsch

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Italiano

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Español

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Swedish

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1. ECL™ Semi-dry blotters: Description

The TE 70, TE 77, TE 70 PWR, and the TE 77 PWR semi-dry blotters rapidly transfer proteins from polyacrylamide gels onto a membrane by means of a low current and low voltage electrotransfer with minimal Joule heating. Most transfers are complete in one hour or less.

The smaller TE 70 unit transfer surface is 14 × 16 cm, suitable for transferring standard gels, including those from the SE 600 Ruby and the SE 400. The larger TE 77 transfer surface is 21 × 26 cm, suitable for transferring gels from the Ettan DALT systems.

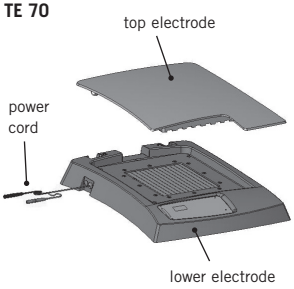
The TE 70 and TE 77 have safety circuits built into the instrument that limit the voltage to 30 V and the current to 0.5 A. The circuits protect the user from unnecessary electrical hazards.

The TE 70 PWR and the TE 77 PWR have a built-in power supply for transferring gels. This eliminates the need for an external power supply. These instruments can deliver up to 30 V and 0.5 A. The instruments also monitor the transfer stack resistance, and can stop a transfer if large changes indicate that the buffer system is depleting.

Multiple gels can be transferred at the same time by placing several small gels of the same thickness side by side, or by stacking two gels vertically in a carefully constructed multi-layered stack.

The electrodes are made of the best possible materials to ensure the units last a long time.

TE 70



TE 77 PWR

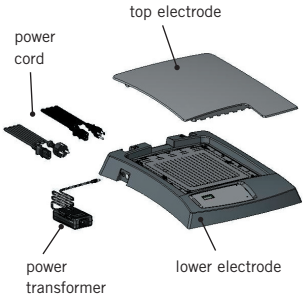


Fig 1. Semi-dry transfer unit main components.

The TE 70 and TE 77 require a power supply with a minimum rating of 0–500 mA, 0–30 V.

Unpacking

Unwrap all packages carefully and compare contents with the packing list, making sure all items arrived. If any part is missing, contact Amersham Biosciences. Inspect all components for damage that may have occurred while the unit was in transit. If any part appears damaged, contact the carrier immediately. Be sure to keep all packing material for damage claims or to use should it become necessary to return the unit.

This declaration of conformity is only valid for the instrument when it is:

- used in laboratory locations.
- used as delivered from Amersham Biosciences except for alterations described in the user manual.
- connected to other CE labeled instruments or products recommended or approved by Amersham Biosciences.

Specifications

	TE 70	TE 77	TE 70 PWR	TE 77 PWR
Electrode size (cm)	14 × 16	21 × 26	14 × 16	21 × 26
Output:				
Voltage	30 V	30 V	30 V	30 V
Current	0.5 A	0.5 A	0.5 A	0.5 A
Inputs:				
Voltage			100–240 V	100–240 V
Current			0.7 A	0.7 A

Minimum transfer stack thickness:	
TE 70, TE 70 PWR	3.2 mm
TE 77, TE 77 PWR	2.8 mm
Environmental operating conditions:	Indoor use: 4–40 °C Humidity up to: 80% Altitude up to: 2000 m Installation category: II Pollution degree: 2
Dimensions (w × d × h):	38 × 46 × 9 cm (15 × 18 × 3.5 in)
Weight:	Shipping 6.8 kg Unit 3.7 kg
Certification:	EN61010–1, EN 61326, CE

Important information

English

- The electric components in the transfer unit base must not become wet. Do not immerse the unit in water. Rinse only the electrodes with distilled water before and after use. (Refer to the Care and maintenance section for cleaning instructions.)
- Be sure to use enough buffer-soaked sheets of blotting paper on both sides of the membrane/gel stack so that the buffer does not become depleted during the transfer.
- If this equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.
- Only accessories and parts approved or supplied by Amersham Biosciences may be used for operating, maintaining, and servicing this product.

Informations importantes

Français

- Les composants électriques dans la sûreté enclenchent le logement de la base d'unité de transfert ne doit pas devenir mouillé. Ne pas immerger de partie de l'unité dans l'eau. Rincer seulement les électrodes avec l'eau distillée avant l'usage. (Se référer à la section de Soin et entretien pour nettoyer d'instructions.)
- Etre sûr d'appliquer assez de tampon feuilles trempées de tacher de papier sous la membrane et par-dessus le gel pour empêcher la pile du séchage hors. Les dommages irréparables à l'unité résulteront si la pile sèche hors et l'unité est permise de surchauffer.
- Si cet équipement est utilisé dans une manière pas spécifié par le fabricant, la protection fournie par l'équipement peut être altérée.
- Seulement les accessoires et les parties ont approuvé ou fourni par Amersham Biosciences sont recommandés pour l'utilisation, l'entretien et réparation de cet appareil.

2. Operating instructions

To transfer proteins, prepare the unit, assemble the stack, and connect to a power supply if necessary. Then run the transfer for the required amount of time. Each step is described below.

Assemble the transfer stack

❶

Prepare the unit by rinsing the anode and cathode with distilled water.

❷

Prepare the gel

Cut away the wells and/or stacking gel section. Equilibrate the gel in transfer buffer if required.

❸

Prepare the transfer stack

Cut the blot paper and transfer membrane to the same size as the gel. Stack the layers carefully so the edges align. If for some reason the membrane needs to be larger than the gel, use a mylar mask (see optional step below) to ensure the current does not bypass the gel.

Optional: **Cut a mask to the proper size**

Measure the gel. Cut an opening centered in the solid mask to a size roughly 2 mm smaller than the gel. Take care with sharp blades when cutting the masks. Place the mask in the base of the unit, centering the opening.

Note: Take care to place the gel correctly on the first try because proteins begin to transfer immediately; once transfer has begun, moving the gel will distort results or cause "shadow" bands on the blot.

4

Prepare the blotting paper

For each gel, cut at least 6 pieces of blotting paper the *same size as the gel* or *slightly smaller.*

Gauge the thickness or number of blotting paper layers according to the amount of buffer required; up to 300 ml of buffer may be required for larger gels or for transfers of 60 minutes in order to prevent the stack from drying out or the buffer from becoming depleted.

Note: Each 21 × 26 cm blot paper will absorb approximately 50 ml of transfer buffer. Each 14 × 16 cm blot paper will absorb 20 ml of buffer.

Saturate at least 3 pieces of the blotting paper with transfer buffer. One by one, center each sheet on the lower electrode and remove all trapped air by rolling a clean pipet or roller from the center toward the edges.

5

Prepare the membrane

Hybond membranes are recommended. (See related products section).

Note: Always wear gloves when handling membranes to avoid leaving fingerprints.

For each gel, cut 1 membrane the same size as the gel or slightly smaller. (A larger membrane may contact an electrode panel, creating a pathway by which current can bypass the gel.)

Pre-wet nitrocellulose or nylon membranes with distilled water. Pre-wet PVDF or other hydrophobic membranes with methanol. Then soak all membrane types in transfer buffer for 2–5 minutes.

Important! Stack each layer with care, with edges parallel. As each layer is added, remove all air pockets by rolling a clean pipet from the center to the edges. Add a few drops of buffer to trouble areas to help remove air pockets.

6

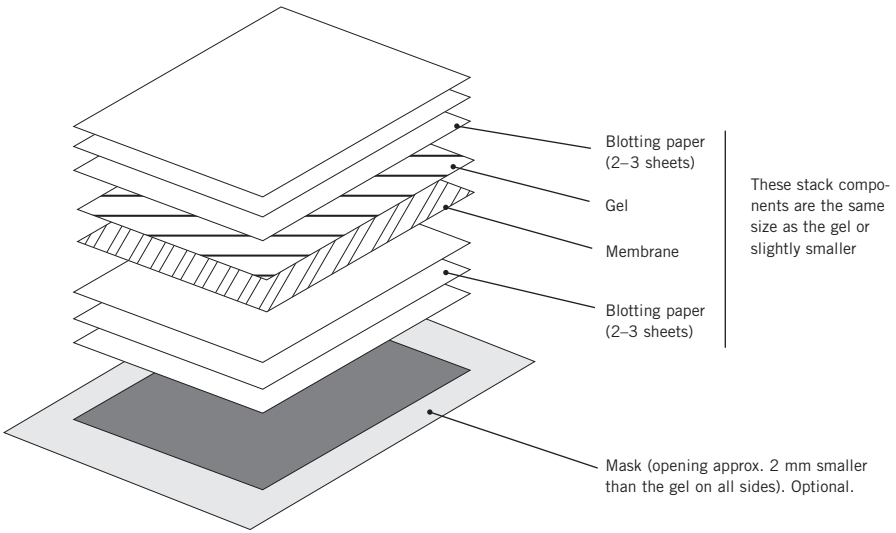
Complete the stack

a) Place the pre-wet membrane onto the stack of blotting paper.

b) Place the gel on the membrane. **Note:** Proteins bind to the membrane as soon as contact occurs, so it is important to place the gel correctly on the first try.

c) Cover the gel with three layers of saturated blotting paper.

Fig 2. Transfer stack for a single gel.



Note: When transferring multiple gels, transfer efficiency depends on such factors as gel thickness, gel position in the stack, transfer buffer, membrane type, and, most importantly, the characteristics of the protein. The gel closest to the anode generally transfers the most completely. It is preferable to lay gels side-by-side rather than stacking them.

Multiple gels: Either lay gels of the same thickness side-by-side (Fig 3), or stack 2 sandwiches layered as shown (Fig 4).

For best results, the transfer stack should be centered in the electrode panels.

If two gels are stacked, separate them with porous cellophane — **not plastic wrap!** (Cellophane permits electric current to pass but stops proteins.) Cut the cellophane slightly smaller than the gel and wet with transfer buffer. Several sheets of buffer-soaked blotting paper on *each* gel provides electrical continuity.

Place the Cover on top of the transfer stack. Do not remove the cover until after the transfer is complete in order to prevent stack components from moving.

Fig 3. Transfer stacks for gels placed side-by-side.

Note: There must be no electrical contact between the two stacks.

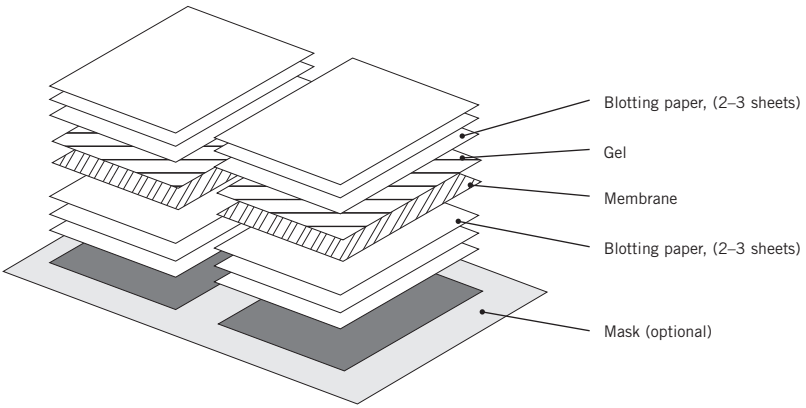
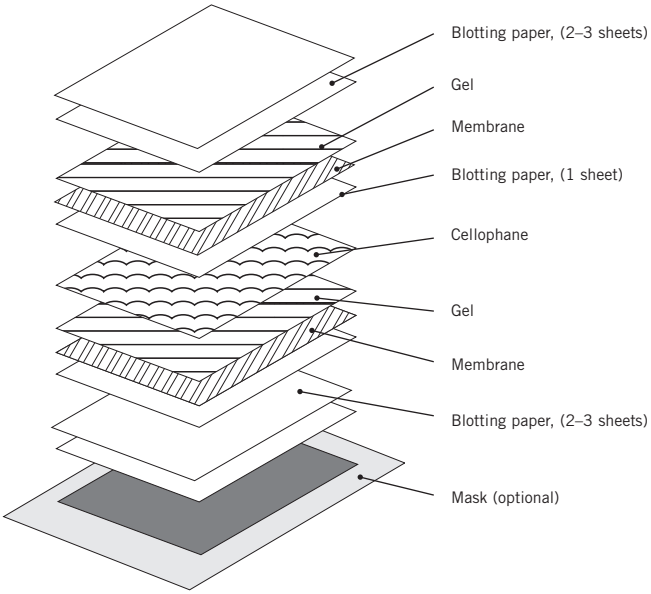


Fig 4. Transfer stack for stacked gels.

Separate the stack for each gel with a sheet of cellophane.



Electrotransfer

TE 70 and TE 77

Note: Transfers exceeding one hour will require additional sheets of buffer saturated blot paper in the transfer stack.

❶

The power supply should be switched off and both the current and voltage controls set at zero

Then plug the color-coded leads from the base of the transfer unit into the power supply jacks, matching red to red and black to black. Do not reverse polarity. (Red is positive [+] and black is negative [–].)

❷

Set the power supply current

The maximum current setting should not exceed 0.8 mA/cm^2 of the gel surface. If transferring several layers of gels, a longer transfer time may be required. Use the graph below to quickly find the current setting for your gel size, or calculate the gel area (cm^2) and multiply it by 0.8 mA/cm^2.

❸

Note: Generally, smaller fragments transfer more quickly than larger ones.

If available, set the power supply timer

Most transfers are complete within one hour, but larger proteins, proteins from native gels, and thicker gels may require an additional 1 hour of transfer time. The optimum transfer time for each protein and gel system must be determined empirically.

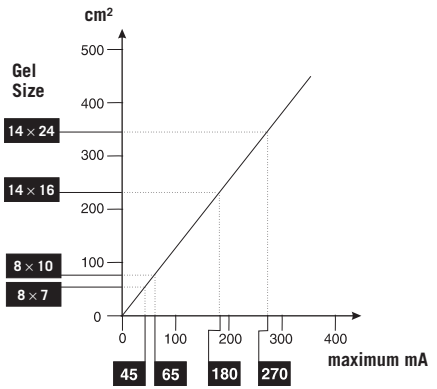


Fig 5. Recommended current settings for different gel sizes.

TE 70 PWR and TE 77 PWR

Turn the instrument on using the POWER button on the front keypad.

The instrument works by setting the current and time, and then starting the transfer. During the transfer, the voltage can be displayed. The transfer can not be set to run at constant voltage.

Note: While setting the time, there is an extra setting labelled "on" above the 4:00 upper limit. This will disable the timer, allowing the transfer to run continuously until manually turned off by the user.

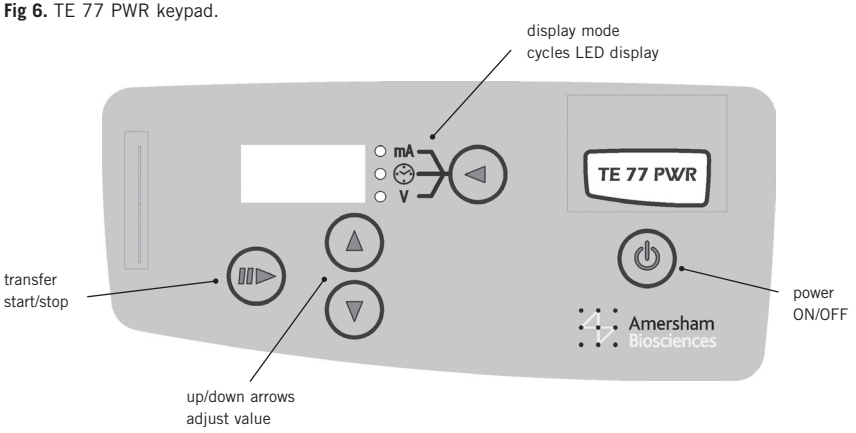
The DISPLAY MODE BUTTON toggles between the current (mA), time (hour:minutes) and the voltage (V).

The Up and DOWN arrows to change the value for the current and time.

Press the START/STOP button to start the transfer.

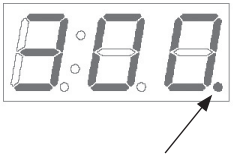
Variable	Units	Range	Increment
Current	milliamps	1–500	1 mA
Time	hours:minutes	5 min – 4:00 hours	5 min
Voltage	volts	0–30 (read only)	1 volt

Fig 6. TE 77 PWR keypad.



During the transfer

TE 70 PWR and the TE 77 PWR



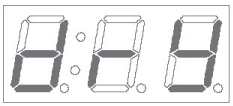
A red LED will appear in the lower right corner of the Display when voltage is applied to the transfer stack.

Both the Time and the Current can be changed as the transfer progresses. Select mA or time using the DISPLAY MODE button, and the UP and DOWN to change the value. While the value is being changed, the LED will blink. After about 10 seconds the LED will stop flashing and show the real time run parameters.

Buffer Depletion

One of the more common failure modes for a Western transfer is buffer depletion. Buffer depletion leads to changes in pH and overheating, both of which are detrimental to the transfer.

The TE 70 PWR and the TE 77 PWR instruments monitor the transfer stack resistance. Large changes in this resistance indicate the depletion of the buffer system. The instrument can stop a transfer before these changes lead to further problems, like burning of the transfer stack.



Dry error message.

If this condition happens, the error message “dry” will be on the display. The instrument has stopped the transfer. If desired, the cover can be removed, and buffer can be added to the transfer stack. Replace cover, and press any key (except Power ON/OFF) to continue.

For future transfers, add more layers of buffer soaked blot paper.

After the transfer is complete

TE 70 and TE 77

Turn off the power supply and disconnect the leads from the power supply.

TE 70 PWR and TE 77 PWR

After a transfer is complete, the unit will beep for 5 seconds. The LED display will blink and cycle between the values for the current, time and voltage at the time the transfer was stopped. These can be recorded into a notebook, if desired. Pushing any button will clear the display.

Note: Staining the gel(s) for residual protein gives an indication of the completeness of transfer.

Note: Rewet dried membranes in the appropriate wetting buffer prior to processing.

❶

Remove the cover slowly because the stack may adhere to it.

❷

Remove and dispose of the upper blot papers.

❸

Remove the gel(s).

Optional: Stain gel to check for residual protein left in the gel.

❹

If desired, label the gel contact side of the membrane with a soft pencil.

❺

Remove the membrane(s) from the stack with blunt forceps. Process the membrane according to your protocol or allow the membrane to air dry prior to storage.

❻

Remove the remaining blot papers and dispose.

❼

Rinse the unit according to the Care and maintenance instructions (page 15).

ECL reagents

Specific and sensitive Western blotting detection on Hybond membranes can be achieved with the ECL Western blotting systems. (See related products section page 24).

A full range of products is available for Western blotting including ECL, ECL Plus, ECL Advance and ECL Plex Western blotting systems.

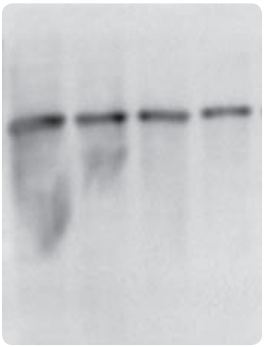


Fig 7. Transfer of *E. Coli* extracts using the TE 77 PWR instrument followed by detection of *E. Coli* GroEL on Hybond P membrane using the ECL Advance detection system. Images were acquired using the Typhoon 9410 in chemiluminescence mode.

3. Care and maintenance

- Do not autoclave or wash the unit in a dishwasher.
- Do not immerse the unit in water!

Rinse the cathode in the cover and the anode in the base with distilled water. Let the unit air dry completely. If using radioactive reagents, decontaminate the unit with a cleaning agent such as Decon™ 90 or Contrad™ 70. Never use abrasive cleansers.

4. Troubleshooting

problem	solution
Incomplete transfer	
Blank or faint areas on the membrane	Remove trapped air pockets between the gel and membrane during stack assembly.
	Use buffer with a lower ionic strength.
Molecules do not migrate out of gel	Check all electrical connections. Confirm that current is flowing through the transfer stack.
	Check that the buffer pH is close to the intended pH. Most buffers should not be titrated. Make fresh buffer.
	Use 3.5 mM SDS (0.1%) in the transfer buffer.
	Add several more sheets of buffer-saturated blotting paper to each side of the gel sandwich so that more buffer is present during the transfer.
	Increase the transfer period. Large fragments may require an additional hour.
	Do not use staining or fixing agents on the gel before transfer.
	Use a thinner gel.
	Reduce the gel acrylamide concentration.
	If using a non-nitrocellulose membrane, avoid including methanol in the transfer buffer or reduce the amount to the minimum possible.
	Use reagent-grade chemicals.
	Increase the net charge on the protein by using a transfer buffer with a different pH. Lower pH (<6–7) increases the positive charge on proteins; higher pH (>6–7) increases the negative charge on proteins.

problem	solution
Open circuit or no output current for TE 70 or TE 77	Blown fuse in protection circuit. Replace fuse.
Smeared or diffuse band patterns	If equilibrating before the transfer, shorten or eliminate the equilibration time and/or equilibrate under coldroom conditions.
	If the transfer buffer contains ≥10% methanol, equilibrate the gel in transfer buffer for 30 minutes to allow it to shrink before assembling the stack. **Note:** Large proteins may not migrate as readily once the pore size is slightly reduced.
	Take care that the gel does not shift once it contacts the membrane.
	Buffer depletion can change the pH in the transfer stack, and have a negative effect on the transfer. On subsequent transfers, either shorten the transfer time, reduce the current, or increase the number of buffer-soaked blotting papers in the stack.
	Check that the preferred binding surface of the membrane (if any) contacts the gel.
Uneven band transfer	The blotting paper and membrane must be the same size as the gel or 1–2 mm smaller. Larger sizes will provide an electrical path for current to bypass the gel solution.
	Different proteins will transfer at different rates depending on size and net charge.

problem	solution
Inefficient binding to membrane	
Chemical parameters	Prepare transfer buffer without SDS. (SDS can improve transfer efficiency but can interfere with protein binding to a PVDF membrane.)
	Add 10–20% methanol to the transfer buffer to enhance binding to nitrocellulose or PVDF.
Membrane parameters	Use a membrane with a smaller pore size (0.20 µm) if proteins pass through the membrane.
	Place a membrane both over and under the gel to capture any proteins migrating in the opposite direction.
	Check if too much sample is available for the binding surface area by placing two membranes instead of one. If "blow through" occurs, reduce the sample load.
	Wear gloves when handling membranes.
	Store membranes at ambient temperature and out of direct sunlight.
	Check shelf life of nitrocellulose membrane, replace if necessary.
TE 70 PWR, TE 77 PWR	
Current reading lower than setting	Maximum voltage (30 V) has been reached. Buffer may be depleted.
No output voltage	Transfer stack is not making contact with the upper electrode. Add buffer soaked blot papers.
"dry" error message	Instrument has detected large changes in transfer stack resistance.
	Add more buffer soaked blot papers in future transfers.

Note: For more troubleshooting ideas, refer to Bjerrum, O.J. *et al.* (1988).

5. Electrotransfer notes

- Run the transfer as soon as possible after electrophoresis to minimize protein diffusion within the gel.
- Stacked gels must all be the same size.
- Limit transfers to two hours or less.
- The recommended methanol concentration for different membrane types are:

membrane type	methanol %
Charged nylon	0
Nitrocellulose	10–20
PVDF	10–20

- Use a buffer with low ionic strength such as one of the two listed below to prevent overheating. Use the CAPS buffer when Tris cannot be used (*e.g.*, peptide sequencing). CAPS can improve transfer because of its effect on the charge of the protein (see Matsudaira, 1987).

Note: Buffers containing methanol may deteriorate if stored for long periods — add methanol just prior to transfer.

Towbin buffer

(25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3, 1 liter)

Tris (FW 121.1)	25 mM	3.0 g
Glycine (FW 75.07)	192 mM	14.4 g
SDS* (FW 288.4)	0.1% (3.5 mM)	1.0 g

Dissolve in 600 ml distilled water.
Add methanol as required†.
Bring to 1 liter with distilled water. Do not adjust the pH, which should be between 8.2–8.4.
Optional: Chill before use.

**Optional:* Adding SDS can improve transfer efficiency.

†Depending on the membrane type selected (see table above), adding methanol can improve transfer results.

CAPS buffer, 1X

(10 mM CAPS, pH 11.0, 1 liter)

CAPS (FW 221.3) [3-(cyclohexylamino)-1-propanesulfonic acid]	10 mM	2.2 g

Dissolve in 600 ml distilled water, adjust to pH 11.0 with conc. NaOH.
Adjust volume to 1.0 liter.

- For a 3-buffer system, refer to Kyhse-Anderson, J. (1984).
- Transfer efficiency varies depending on the gel concentration, which can be optimized. For more information, refer to Smejkal and Gallagher (1994).

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7. Customer service information

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8. Ordering information

product	qty.	order no.
TE 70 Semi-dry Transfer Unit, 14 × 16 cm Includes 25 sheets of blotting paper, 50 sheets of cellophane, 2 solid masks, masks for 7 × 8 cm and for 14 × 16 cm gels	1	80-6210-34
TE 77 Semi-dry Transfer Unit, 21 × 26 cm Includes 25 sheets of blotting paper, 50 sheets of cellophane, 2 solid masks, mask for 14 × 16 cm gels	1	80-6211-86
TE 70 PWR Semi-dry Transfer Unit, 14 × 16 cm Includes 25 sheets of blotting paper, 50 sheets of cellophane, 2 solid masks, masks for 7 × 8 cm and for 14 × 16 cm gels	1	11-0013-41
TE 77 PWR Semi-dry Transfer Unit, 21 × 26 cm Includes 25 sheets of blotting paper, 50 sheets of cellophane, 2 solid masks, mask for 14 × 16 cm gels	1	11-0013-42

Accessories

TE 70 and TE 70 PWR		
Solid masks 16.5 × 18.5 cm	4	80-6210-72
Porous cellophane, 20 × 35.5 cm	50	80-6210-53
Blotting paper, precut, 14 × 16 cm	25	80-6211-67
TE 77 and TE 77 PWR		
Solid masks 23 × 27.5 cm	4	80-6212-05
Porous cellophane, 35 × 44 cm	50	80-6117-81
Blotting paper, precut, 21 × 26 cm	25	80-6211-29
Power Cord Kit	1 set	28-4025-98
ECL Semi-dry blotters User Manual (*this manual*)	1	28-4025-91
Fuse 1.6 A, 250 V, SB 5 × 20	5	80-6108-69

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product	order no.
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MiniVE Vertical Electrophoresis system	80-6418-77
EPS 2A200	80-6406-99

Transfer Membranes

Hybond ECL pure Nitrocellulose, sheets and rolls	
0.45 µm pore size	
Hybond ECL 7 × 8 cm, 50 sheets	RPN78D
Hybond ECL 15 × 20 cm, 10 sheets	RPN1520D
Hybond ECL 20 × 20 cm, 10 sheets	RPN1520D
Hybond ECL 30 cm × 3 m, 1 roll	RPN303D
0.2 µm pore size	
Hybond ECL 30 cm × 3 m, 1 roll	RPN3032D

Hybond-P, PVDF membrane, sheets and roll	
0.45 µm pore size	
Hybond-P, 20 × 20 cm, 10 sheets	RPN2020F
Hybond-P, 14 × 16 cm, 15 sheets	RPN1416F
Hybond-P, 30 cm × 3 m, 1 roll	RPN303F

Hybond-LFP, low fluorescent PVDF membrane, sheets and roll	
0.45 µm pore size	
Hybond-LFP, 20 × 20 cm, 3 sheets	RPN2020LFP3
Hybond-LFP, 20 × 20 cm, 10 sheets	RPN2020LFP
Hybond-LFP, 14 × 16 cm, 15 sheets	RPN1416LFP
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ECL Mouse IgG, HRP-Linked Whole Ab	NA931-1ML
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ECL Western blotting detection reagents	RPN2109
ECL Plus Western blotting detection reagents	RPN2132
ECL Advance Western blotting detection kit	RPN2135
ECL Plex goat-α-mouse IgG, Cy™3, 600 µg	PA43010
ECL Plex goat-α-mouse IgG, Cy5, 600 µg	PA45010
ECL Plex goat-α-rabbit IgG, Cy5, 600 µg	PA45012
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ECL Plex Combination Pack, Hybond ECL	RPN998
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