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Полусухой блоттер SEMIDRY

Semi-Dry Blotters

Instruction Manual

Catalogue Numbers

SD10

SD20

Safety Information

When used correctly, these units pose no health risk. However, these units can deliver dangerous levels of electricity and are to be operated only by qualified personnel following the guidelines laid out in this instruction manual. Anyone intending to use this equipment should read the complete manual thoroughly. The unit must never be used without the safety lid correctly in position. The unit should not be used if there is any sign of damage to the external tank or lid.

These units comply with the following European directives:

2006/95/CE Low Voltage Directive and 2014/30/UE (official Title 2004/108/EC) EMC Electromagnetic Compatibility

By virtue of the following harmonised standards:

BS EN IEC 61010-1: 2010 Safety Testing of Lab Equipment

BS EN IEC 61326-1:2013 EMC Electro Magnetic Compatibility

Packing List

Each multiSUB unit includes a tank, wired electrodes, lid and the following items:

SKU	Main Unit Base & Lid	CS-SDCAB (Cables)	Instruction Manual

The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received.

Cleaver Scientific is liable for all missing or damaged parts / accessories within 7 days after customer received this instrument package. Please contact Cleaver Scientific immediately regarding this issue. If no response within such period from consignee party, that will be consignee party's whole responsibility.

Please contact your supplier if there are any problems or missing items.

detergents. If the salts are not removed, they will increase the conductivity of the transfer buffer and the amount of heat generated during the transfer. Also, low percentage gels (i.e. <12% acrylamide) will shrink in methanol-containing buffers. Equilibration allows the gel to adjust to its final size prior to electrophoretic transfer.

2. Cut the membrane and filter papers to the size of the gel.

Note: 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 protein contamination.

3. Wet the membrane by slowly sliding it at a 45° angle into transfer buffer and allowing it to equilibrate for 15–30 minutes. Complete wetting of the membrane is important to insure proper binding. Abrupt wetting can lead to entrapment of air bubbles in the matrix. These air bubbles can block transfer of molecules. Ensure that excess liquid drains from the membrane.
4. Soak the required number of filter paper pieces into the transfer buffer (generally two pieces of extra thick filter papers or four pieces of thick filter papers or six pieces of thin filter papers). Typical buffers for different types of blotting are listed in the specified section.
5. Mark with a pencil or use some other means to enable identification of the gel side of the membrane. This is necessary for the blot probing stage; more efficient binding can occur when the membrane is facing upwards in the probe solution. It is also an idea to clip the corner of the gel nearest to the top where lane 1 was on the gel to allow easy sample identification during analysis.
6. Remove the lid from the blotter and place 1 extra thick pre-soaked filter paper onto the base electrode plate ensuring that any excess liquid is wiped away and remove air trapped using a roller.
7. Carefully place the membrane on top of the filter paper and ensure that no air pockets have formed. Any air pockets should be smoothed out using a roller.
8. Place the gel on top of the membrane and smoothen with a roller 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 adhere to the gel evenly.
9. Place the remaining filter paper pad on top of the membrane and roll out any trapped bubbles gently.

10. 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 be disturbed if one screw is tightened fully, then the next.

Note: only use the screws for blotting of acrylamide gels up to 2mm thick. Do not overtighten the screws as this could cause dry out of the blotting sandwich and affect the transfer. For blotting thicker gels and agarose gels do not use the screws. The weight of the lid will provide enough pressure.

11. 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. (Please see below)

12. Attach the power leads to the appropriate sockets, red to red, black to black on a power supply.

Note: Do not invert the leads or connect up incorrectly as this will cause corrosion of the stainless-steel electrode.

13. Blotting generally requires high current settings >250mA and the power supply should contain these capabilities. Please contact your Cleaver Scientific representative for details of these.

Running the Blot

Transfer condition will vary according to specific protocol, however, please find below suggested settings:

- Transfer mini gels for 15–30 minutes at 10–15 V (SD10 or SD20).
- Large gels can be transferred for 30 minutes to 1 hour at 15–25 V (SD20).

A current limit (3 mA/cm2 for large gels; 5.5 mA/cm2 for mini gels) is recommended to prevent excessive heating during the run. Transfers may not always be quantitative; therefore, it is always recommended to run a positive control for comparative analysis. It is generally best to perform a time course for the type of gel, samples and buffer being used to optimize transfer efficiency.

The efficiency and quality of transfer depends on the type of buffer used and on the type of samples. Better results are usually obtained by reducing the power settings and increasing the blot time (Transfers longer than 2 hours are not recommended).

Solutions

NOTE: Transfer buffers must be made accurately using high grade reagents. Do not adjust the pH with acid or base as this will affect the properties of the buffer. pH will vary according to the purity of the reagents used. Improperly prepared buffer will cause excess heat generation and safety hazards

Western Blotting buffers

Bjerrum and Schafer-Nielsen transfer buffer

48 mM Tris, 39 mM glycine, (20% methanol) pH 9.2

- Dissolve 5.82 g Tris and 2.93 g glycine in dH2O (add 200 ml of methanol); adjust volume to 1 litre with dH2O
- N.B SDS may be added to increase protein elution from the gel:

48 mM Tris, 39 mM glycine, (20% methanol), 1.3 mM SDS (0.0375%), pH 9.2

- Dissolve 5.82 g Tris and 2.93 g glycine, and 0.0375 g SDS or 3.75 ml of 10% SDS in dH2O (add 200 ml of methanol); adjust the volume to 1 litre with dH2O

Towbin transfer buffer

25 mM Tris, 192 mM glycine (20% methanol), pH 8.3

Dissolve 3.03 g Tris and 14.4 g glycine in dH2O (add 200 ml of methanol); adjust volume to 1 litre with dH2O

Dunn carbonate transfer buffer

10 mM NaCHO3, 3 mM Na2CO3 (20% methanol), pH 9.9

Dissolve 0.84 g NaHCO3 and 0.318 g Na2CO3 (anhydrous) in dH2O (add 200 ml of methanol); adjust volume to 1 litre with dH2O.

Southern/Northern blotting buffers

TBE buffer for nucleic acid transfer

5x TBE stock solution 0.5 M Tris, 0.5 M boric acid, 10 mM EDTA in dH2O

- Dissolve 54 g Tris base, 27.5 g boric acid and 20 ml 0.5 M EDTA, pH 8.0; adjust volume to 1 litre with dH2O.
- Dilute to 0.5x TBE with dH2O for the working solution (transfer buffer).

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3. Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience,1989.
4. Dunbar, B.S., Ed. 1994. Protein Blotting: A Practical Approach. IRL Press at Oxford University Press, Oxford, England.
5. Hybond Blotting Guide: The direct route to excellent blotting results", Amersham Life Science.
6. Sambrook, Fritsch, and Maniatis, Molecular Cloning A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989.

Tovey, E.R. and B.A. Baldo. 1987. Comparison of semidry and conventional electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes. Electrophoresis 8: 384-387.

Troubleshooting

<table>
<tr><th colspan="2">Nucleic Acids</th></tr>
<tr><td>Poor nucleic acid transfer</td><td>Transfer apparatus assembled incorrectly and proteins moving in the wrong direction
Gel/membrane sandwich may be assembled in the wrong order, or cassette inserted in wrong orientation. Check polarity.</td></tr>
<tr><td>DNA / RNA remains within gel</td><td>Gel too hot and buffer too concentrated, resulting in excessive current and the gel starting to melt. Remake buffer to 0.5X TBE, the concentration required for proper transfer
Power conditions changed during transfer. Important to maintain constant current. If buffer less concentrated than 0.5X, 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’.</td></tr>
<tr><td>Poorly blotted or diffused transfer</td><td>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.</td></tr>
<tr><td>Poor Detection Sensitivity</td><td>Poor DNA probe labelling
Insufficient signal hybridized to target DNA for detection. Labelled DNA probe not properly labelled. Check labelling 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.</td></tr>
</table>

<table>
<tr><td></td><td>Hybridization conditions may be too stringent; alter to reduce stringency and improve efficiency of probe-template binding.</td></tr>
<tr><td>High Background</td><td>Increase hybridization stringency to reduce non-specific probe binding.</td></tr>
<tr><td colspan="2">Protein</td></tr>
<tr><td>Poor protein transfer</td><td>Transfer apparatus assembled incorrectly and proteins moving in the wrong direction.
Gel/membrane sandwich may be assembled in the wrong order, or cassette inserted in wrong orientation. Check polarity.
Air pockets not removed while assembling the blotting sandwich</td></tr>
<tr><td></td><td>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.</td></tr>
<tr><td></td><td>Transfer time too short – increase transfer time</td></tr>
<tr><td></td><td>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 550mA for SD10 or 1200mA for SD20
Buffer may be prepared improperly – prepare new buffer and increase voltage.</td></tr>
<tr><td></td><td>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.</td></tr>
<tr><td></td><td>Defective or inappropriate power supply used.
Check fuse of power supply. Ensure max. current output of power supply is at least 2000mA.</td></tr>
<tr><td></td><td>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.</td></tr>
<tr><td>Protein precipitating in gel</td><td>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.</td></tr>
<tr><td>Swirls or missing bands; diffuse transfers</td><td>Poor gel-membrane contact. Air bubbles or excess buffer remain between membrane and gel.</td></tr>
</table>

	• Carefully remove air bubbles between gel and membrane using a rolling pin • Use more, or thicker, filter paper in gel-membrane sandwich • Replace the fibre pads, as they degrade and remain permanently compressed over time.
	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.
Gel cassette pattern transferred to blot	Contaminated fibre pads • Replace fibre pads or clean thoroughly. Contaminated transfer buffer • Replace buffer solutions.
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 (0.2µm) 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 <15kDa have reduced binding to 0.45µm 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 2000mA.
Immune-specific detection	Overall high background • Reduce antibody / protein sample concentration Too low background • Increase antibody concentration / protein sample concentration Consult manual included with antibody detection kit
Total protein detection	Consult stain or detection kit manual.

Care and Maintenance

Cleaning the Semi Dry Blotting Units

Units are best cleaned using warm water and a mild detergent. Water at temperatures above 60^0 C can cause damage to the unit and components.

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 preferable before use.

The units should only be cleaned with the following:-

Warm water with a mild concentration of soap or other mild detergent.

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.

RNase Decontamination

This can be performed using the following protocol:-

Clean the units with a mild detergent as described above.

Wash with 3% hydrogen peroxide (H2O2) for 10 minutes.

Rinsed with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,

Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using. RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

Warranty

The Cleaver Scientific Ltd. (CSL) Semi Dry units have a warranty against manufacturing and material faults of twelve months from date of customer receipt.

If any defects occur during this warranty period, CSL will repair or replace the defective parts free of charge.

This warranty does not cover defects occurring by accident or misuse or defects caused by improper operation.

Units where repair or modification has been performed by anyone other than CSL or an appointed distributor or representative are no longer under warranty from the time the unit was modified.

Units which have accessories or repaired parts not supplied by CSL or its associated distributors have invalidated warranty.

CSL cannot repair or replace free of charge units where improper solutions or chemicals have been used. For a list of these please see the Care and Maintenance subsection.

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