nUView Precast Gels

WESTERN BLOTTING PROTOCOL

The following blotting procedures are recommended for all NuSep Gels.

It is recommended to read apparatus manual before conduction blots as varying blotting conditions will alter results.

It is recommended that you use NuSep Transfer Buffer BG-168.

The following recipe can also be used:

Tris Base	3.00 g
Bicine	4.08 g
Ethanol or Methanol	100 mL
MilliQ™ water to (approx. pH 10.5)	1000mL

NOTE:
Addition of 0.05% SDS will improve the transfer of protein out of the gel however this reduces the ability for proteins to bind to nitrocellulose membranes.

It is recommended to use SDS with PVDF membranes only.

Wet Blotting Protocol

1. Cool the transfer buffer to 4˚C.
2. Equilibrate the gels in transfer buffer for 5 minutes.
3. Soak Scotch-Brite™ pads, filter papers and membranes in Transfer Buffer (BG-168) for 5 minutes. PVDF membranes must be wetted in methanol before equilibrating in Transfer Buffer (BG-168).
4. Assemble the transfer sandwich as follows (ensure there are no air bubbles particularly between gel and membrane):
   - Cathode (----)
   - Scotch-Brite™ pad
   - 2x filter paper
   - Gel
   - Transfer Membrane
   - 2x filter paper
   - Scotch-Brite™ pad
   - Anode (+++)

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5. The blotter should be firmly packed but not too tight as to squeeze the transfer buffer from the wetted filter paper and Scotch-Brite™ pads.
6. If two membranes are to be blotted, repeat the above transfer sandwich. If only one gel is to be blotted, fill the space with more filter paper and another Scotch-Brite™ pad.
7. Pour Transfer Buffer (BG-68) through the sandwich and place into the apparatus.
8. Fill the apparatus with chilled Transfer Buffer (BG-168).
9. Transfer at 40 Volts for 120 minutes.
10. Gently remove gel from sandwich and rinse with transfer buffer.
11. If any gel is adhered to the membrane that needs to be removed soak membrane in RO water until gel is soft, then use cotton wool buds to remove from membrane.

Semi-Dry Blotting Protocol

1. Do not cool the Transfer Buffer (BG-168).
2. Soak the filter papers, membranes and gel in Transfer Buffer (BG-168) for 15 minutes.
3. Assemble the transfer sandwich as follows:
   - Cathode (—-)
   - Filter paper (extra thick filter paper is used in semi-dry blotting, although a stack of thinner paper may be used)
   - Gel
   - Transfer Membrane
   - Filter paper
   - Anode (+++)
4. Transfer the blot for 30 minutes at 20 V.
5. Remove the gel from the sandwich and rinse with transfer buffer.
6. If any gel is adhered to the membrane that needs to be removed soak membrane in RO water until gel is soft, then use cotton wool buds to remove from membrane.

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