



Western Blotting Protocol for NuSep Gels

Note: All standard blotting procedures may be used with NuSep Gels.

Transfer Buffer Recipes

	Tris-Tricine SDS	Tris-HEPES SDS	Tris-Glycine SDS
Tris base	3.00g	3.00g	3.00g
Bicine	4.08	4.08	-
Glycine	-	-	14.40g
Boric Acid	-	-	-
EDTA	-	-	-
Ethanol or Methanol	100mL	100mL	200mL
Milli Q to	1000mL	1000mL	1000mL
Transfer times are for buffers cooled to 4 degrees			
At 40 Volts	90 min	90 min	240 min

NOTE: Addition of 0.05% SDS will improve the transfer of protein out of the gel.
This may have some effect on binding to nitrocellulose membranes.



Western Blotting Protocol for NuSep Gels Continued

Wet Blotting Protocol

- 1 Do not cool the transfer buffer. If adding SDS, cool the transfer buffer to 4°C.
- 2 Equilibrate the gels in transfer buffer for 5 minutes.
- 3 Cut Whatman filter papers to size (10 x 8cm) and soak in transfer buffer.
- 4 Cut membrane(s) to size (10 x 8 cm) and soak in transfer buffer (PVDF membranes must be wetted in methanol first and then equilibrated in aqueous solution).
- 5 Soak the Scotch-Brite™ pads in transfer buffer.
- 6 Assemble the transfer sandwich as follows:
 - a) Cathode (– – –)
 - b) Scotch-Brite pad
 - c) 2x filter paper
 - d) Gel
 - e) Transfer Membrane
 - f) 2x filter paper
 - g) Scotch-Brite pad
 - h) Anode (+ + +)

Blotter should be firmly packed. 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 through the sandwich and place into the apparatus. Fill the apparatus with transfer buffer.
- 8 Place the apparatus in an ice bath for cooling, unless using cooled buffer.
- 9 Transfer at 40V. Transfer times for each gel type.
- 10 Gently remove gel from sandwich and rinse with transfer buffer.
- 11 Use cotton wool buds to remove adhering gel from the membrane.



Western Blotting Protocol for NuSep Gels Continued

Semi-Dry Blotting Protocol

- 1 Do not cool the transfer buffer.
- 2 Soak the filter paper, membrane and gel in transfer buffer for 15 minutes.
- 3 Make the blotting sandwich in a semi-dry blotting apparatus.
 - Anode (+ + +)
 - Filter paper
 - Membrane
 - Gel
 - Filter paper
 - Cathode (---)

(Note: extra thick filter paper is used in semi-dry blotting, although a stack of thinner filter paper may be used.)

- 4 Transfer the blot for 30 minutes at 20V.
- 5 Remove the gel from sandwich and rinse with transfer buffer.
- 6 Use cotton wool buds to remove adhering gel from the membrane.