

Section XI: Blotting Proteins from Polyacrylamide Gels

Introduction

Protein transfer efficiency in blotting applications is dependent upon multiple factors, including gel percentage, gel thickness, protein size, transfer conditions (e.g., buffer and voltage), and type and quality of membrane.

To achieve optimal transfer efficiency, transfer conditions must be adjusted to address these varying factors.

Choosing the appropriate membrane

Nitrocellulose	PVDF	Nylon
Hydrophobic binding	Hydrophobic binding	Hydrophobic & electrostatic binding
General purpose membrane	SDS tolerant	Stable if baked
Low background	High background	High background
Low strength	High strength	High strength
Becomes brittle if baked	Suitable for protein sequencing	Least suitable for Western transfer

Transfer solutions

Formula for Towbin transfer solution:

Towbin Transfer Solution

1X Working Solution	Amount for 1X Working Solution
25 mM Tris base	30.3 g Tris base
192 mM Glycine	144.1 g Glycine
0.1% SDS	10.0 g
	Adjust volume to 8 liters with distilled water
	Measure, but do not adjust pH; it should be approximately 8.2 to 8.4
20% Methanol	2 L Methanol
	Adjust volume to 10 liters with distilled water

It may be necessary to lower the concentrations of methanol, SDS or both to obtain the optimal balance of transfer and binding efficiency. The table below outlines the effects that SDS and methanol have on protein transfer.

SDS	Methanol
Improves transfer of proteins >60 kDa	Improves binding efficiency
Decreases binding efficiency	Decreases transfer efficiency
Not compatible with nylon membranes	Do not soak gel in transfer buffer prior to blotting
Include 0.1% - 0.2% in transfer buffer	Include 20% in transfer buffer

