

Western Blot Protocol

NOTE: This method has been optimized for use with nitrocellulose membrane and a semi-dry transfer.

Reagent	Details
SEQer™ antibodies	Please visit our website: www.antibodies.sdix.com for more details.
4X Protein Loading Buffer:	0.25 M Tris pH 8.0, 40% Glycerol, 8% SDS, 0.4% Bromophenol Blue, 2.88 M β-Mercaptoethanol
TBST Buffer:	Tris buffered Saline, 0.1% Tween 20, pH 7.4
TBST/2% Skim Milk Buffer:	2% Skim Milk added to TBST Buffer
MagicMark XP Western Protein Standard	Invitrogen
Prestained SDS-PAGE Standards, Low	Bio-Rad
Criterion Tris-HCl Gel, 4-20%	Bio-Rad
Goat Anti Rabbit HRP	Zymed
Reversible Membrane Staining Kit	Memcode
West Femto Substrate	SuperSignal
Luminol Enhancer Solution	SuperSignal
1X Tris-Glycine Running Buffer:	0.25 M Tris Base, 1.9M Glycine, 1% SDS
1X Transfer Buffer:	50 mM Tris Base, 30 mM Glycine, 0.37% SDS, 20% MeOH
Protran Nitrocellulose	Whatman
Filter Papers, Grade 320	Ahlstrom

Electrophoresis and Electroblothing

- 1.0 Prepare protein samples (cell lysates, cell lines, tissues) and protein standard markers for separation according to established procedures.
- 2.0 Perform separation of samples via SDS-PAGE using an appropriate gel type (Bio-Rad Criterion Tris-HCl 4-20% Gels).
- 3.0 Transfer protein onto nitrocellulose using semi-dry transfer process (12 constant volts for 1 hour).
Note: This transfer condition is based on Bio-Rad Transblot SD cell semi-dry transfer unit. The transfer condition may vary depend on the gel percentage, gel size, gel thickness and transfer buffer.
- 4.0 Stain membrane with Memcode Reversible Stain (incubate for 1 minute).
 - 4.1 Rinse membrane with deionized water until protein bands are clearly visible.
NOTE: Protein should be transferred uniformly to the membrane with no abnormalities
 - 4.2 Image membrane with suitable camera (Kodak CCD Imaging System).
- 5.0 Destain membrane (Memcode Eraser Solution).
 - 5.1 Rinse membrane with deionized water until membrane appears blank, except for pre-stained markers if used.
- 6.0 Block membrane (BST/2% Skim Milk Buffer on orbital shaker at 50 RPM for 1 hour).
NOTE: If analysis will not be performed immediately, membranes can be stored (20°C, wrapped in plastic wrap inside a Ziploc bag).

Immunoblotting Procedure

For SEQer™ antibodies, SDI recommended a 1:10,000 dilution as a starting point.

NOTE: The recommended dilution range is 1:5,000 – 1:20,000 based on 1 mg/ml antibody concentration.

- 1.0 Incubate the blot in diluted SEQer antibody (TBST/2% Skim Milk Buffer) for 12-24 hours at room temperature with shaking.
 - 1.1 Wash membrane with 10 mL TBST buffer (10 minutes at 50 RPM), repeat 4 times (longer washes may further reduce background).
- 2.0 Prepare HRP-Goat anti-rabbit IgG (H+L), diluting 1:5,000 (TBST/2% Skim Milk buffer). Add 10 mL of diluted conjugate to each blot. (The dilution range for secondary antibody is based on manufacturer's specification for 1 mg/ml antibody concentration.)
 - 2.1 Incubate with conjugate at room temperature (2 hours at 50 RPM).
 - 2.2 Wash membrane with 10 mL TBST buffer (10 minutes at 50 RPM). repeat 4 times (longer washes may further reduce background).
- 3.0 Membrane Development with Chemiluminescent Substrate: Mix equal volumes of SuperSignal West Femto Stable Peroxide Solution and Luminol Enhancer Solution.
 - 3.1 Add 2 mL of substrate and incubate for 1 minute without shaking.
- 4.0 Capture a digital image of the blot with a CCD Camera for further evaluation.

Troubleshooting for Western Blot Detection:

- 1.0 Excess signal: reduce the secondary antibody conjugate concentration and incubation time, reduce protein load on the gel, reduce film exposure time.
- 2.0 Low signal: increase or optimize primary antibody concentration, increase the secondary antibody conjugate concentration and incubation time, increase protein load onto gel, increase film exposure time.
- 3.0 No signal: optimize transfer conditions for complete transfer of desired proteins, ensure that no sodium azide is present because sodium azide will inhibit HRP activity.
- 4.0 High background: increase washing time, optimize washing condition with high-stringent wash (optimize salt and detergent concentrations).

Troubleshooting for Protein Transfer:

- 1.0 Semi-dry transfer provides quick and convenient transfer. For proteins which are difficult to be transferred or proteins with high molecular weight, tank transfer should be considered.
- 2.0 Avoid using thicker gels (> 1 mm), since it requires longer transfer time with additional optimized condition.
- 3.0 Semi-dry transfer may cause uneven-transfer for proteins with different molecular weights. Small proteins tend to go through the nitrocellulose member quickly. The transfer efficiency for large proteins is relatively low. Tank transfer may be considered if uneven transfer becomes a problem.

For technical support, please email antibodysupport@sdix.com
or call 1.800.481.9737.