LP101 – WESTERN BLOT

This protocol is used to transfer proteins onto a membrane after SDS-PAGE and to detect protein using antibodies.

Materials

PVDF membrane
Whatman filter paper
5-ml and 10-ml pipettes
Ice box
Rocking platform
Methanol
HRP-conjugated secondary antibody
SuperSignal Pierce substrate
Kodak film

Solutions

10x Transfer Buffer, pH8.3: 250 mM Tris base, 1.92 M glycine, 1% SDS, no pH adjusting necessary.

1X Transfer buffer: mix 200 ml ethanol, 100 ml 10X Transfer Buffer, 700 ml distilled water and pre-chilled at 4°C.

10X TBS: 250 mM Tris-Cl, pH8.0; 1.25 M NaCl

Blocking Buffer: 1X TBS, 3% non-fat dry milk, 0.05% Tween 20

Wash Buffer: 1X TBS, 0.05% Tween 20

Stripping Buffer: 0.2 M glycine, pH 2.5, 0.05% Tween 20

A. Preparation of membrane

1. Cut a piece of PVDF membrane.
2. Wet in methanol on a rocker at room temperature (RT) for 5 min. Remove methanol and add 1X Transfer Buffer until ready to use.

B. Membrane transfer

1. Assemble "sandwich" for electrophoretic transfer onto PVDF membrane.
2. Wet the sponges and Whatman filter papers in pre-cooled 1X Transfer Buffer and assemble sandwich in the following order:

black side transfer- sponge - filter paper - gel - membrane - filter paper - sponge – red side transfer
Roll a pipette to remove air bubbles between layers.

3. Fill apparatus with pre-chilled 1X Transfer Buffer. For a Mini-Transblot, transfer for 1 hr at 100 V and constant voltage at 4°C.
4. Block for 1 hr at RT or overnight at 4°C with Blocking Buffer on rocking platform.

C. Antibodies and detection

1. Incubate with primary antibody diluted to 0.5-2 μg/ml in Blocking Buffer using 2 ml in a 15-ml conical tube for membrane strips or 10 ml in container for full-size membrane for 1 hr at RT or overnight at 4°C on rocking platform.
2. Wash 4 x 5 min with Wash Buffer using 5 ml for strip or 20 ml for membrane.
3. Incubate with secondary antibody diluted 1:10,000 (HRP-conjugated anti-rabbit) in Blocking Buffer using 2 ml in a 15-ml conical tube for membrane strips or 10 ml in container for full-size membrane for 1 hr at RT.
4. Wash 4 x 5 min with Wash Buffer using 5 ml for strip or 20 ml for membrane.
5. Incubate with 1 part Sol A/1 part Sol B SuperSignal chemiluminescent substrate using 1 ml for strip or 5 ml for membrane.
6. Expose membrane to Kodak film in the dark for 1 min. Adjust time to 30 s or 5 min as appropriate.

D. Stripping blot

1. Rinse membrane off with Wash buffer.
2. Place membrane into Kapak bag cut to slightly larger size than membrane.
3. Add about 5 to 10 ml Stripping buffer.
4. Remove as much air as possible and seal bag.
5. Immerse into 80°C water bath and incubate for 20 min.
6. Rinse membrane off with Wash Buffer.
7. Block for 1 hr at RT or overnight at 4°C with Blocking Buffer.