# 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.