ReliaTech
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Western Blot (Semidry-Blot)

Western blotting is a widely used method for the identification and quantification of proteins in biological samples. It combines electrophoretic separation, transfer to a membrane and antibody-based detection. The sequence of methods is as follows:

1. Electrophoresis (SDS-PAGE): The proteins are separated according to their size.
2. Transfer: The separated proteins are transferred to a membrane (e.g. PVDF or nitrocellulose).
3. Blocking: Non-specific binding sites on the membrane are saturated with a blocking solution.
4. Antibody incubation: A primary antibody binds specifically to the target protein, a secondary antibody with a detection marker (e.g. enzyme or fluorescence) enables visualization.
5. Detection: The proteins are visualized by chemiluminescent, fluorescent or colour-bound signals.

Western blot is frequently used in biomedical research, diagnostics and biotechnology, e.g. to analyze protein expression, post-translational modifications or disease signatures.

Figure 1: Western Transfer and Protein Detection

A. ANTIBODIES

- ReliaTech´s Antigen specific probing antibody (primary antibody; e.g. human antibody)
- Secondary antibody (e.g. anti-human antibody) conjugated to detection marker (e.g. Alkaline Phosphatase)

B. RECOMMENDED MATERIALS

- SDS-PAGE apparatus and accessories
- Dry Blotting Transfer System incl. accessories (e.g. anode and cathode assembly, power supply)

- A PVDF, approximately the size of the gel, presoaked in Western Transfer Buffer for five minutes

  The membrane should be handled with gloves and clean forceps to avoid contamination with extraneous proteins

- 1x TBST
- Western Transfer Buffer (TOWBIN buffer)
  25 mM Tris, 192 mM glycine, 20% (v/v) methanol (pH 8.3)

Tris base	*3.03 g*
Glycine	*14.4 g*
dH_2O	*500 mL*
Methanol	*200 mL*

  Adjust volume to 1 L with dH_2O
  The pH will range from pH 8.1 to 8.5 depending on the quality of the Tris, glycine, methanol, and dH_2O

- Commercially available detection kit (e.g. alkaline phosphatase conjugate kit)

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1. TRANSFER PROTOCOL

1. Load the protein sample onto a 4-20% Tris-Glycine polyacrylamide gel and run until desired resolution is achieved.

   The electrophoresis can be followed by using pre-stained molecular weight markers.

2. Cut the transfer membrane (PVDF) to gel size; hydrophilize with ethanol and transfer to transfer buffer.
3. Cut the filter paper (Whatman paper) to the size of the gel (2 sheets in total).
4. Soak 1 sheet with transfer buffer and place on the anode.
5. Place the transfer membrane on the filter paper without air bubbles.
6. Place the gel on the membrane.

   Caution: Avoid trapping air bubbles.

7. Soak the second filter paper with transfer buffer and position it on the gel free of air bubbles.
8. Place the cathode on top.
9. Connect the anode and cathode to the power supply.
10. Blot a whole gel for 30-40 minutes at 15 V, two gels for 45-50 minutes at 10 V and half a gel for 25-30 minutes at 15 V.

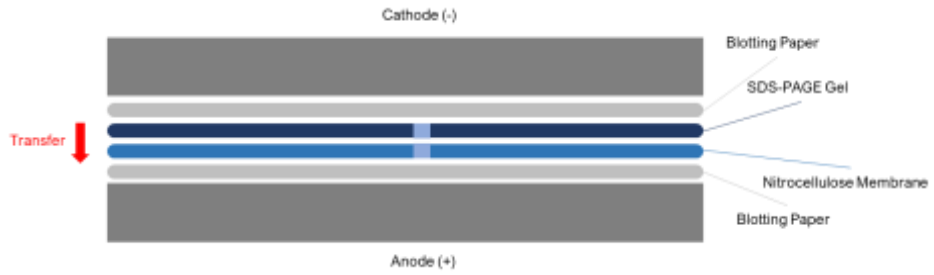

Figure 2: Semidry Blot device

2. IMMUNOSTAINING PROTOCOL

1. After the transfer is complete, incubate the membrane in blocking solution (3% Nonfat Dry Milk in dH_2O) for 40 minutes with gentle agitation on an orbital shaker.
2. Wash the membrane three times with TBST (TBS, pH 7.2 with 0.1 % TWEEN-20) in a clean tray on an orbital shaker; each wash lasting 5-10 minutes.
3. Dilute the probing (primary) antibody in TBST to a volume of 50 mL (approximate final concentration of 0.2 µg/mL) and incubate the membrane in the antibody solution for 1-4 hours at room temperature.

   The optimum incubation time depends on the antibody/antigen binding affinity and must be pre-determined for each antibody

4. Wash the membrane three times as in step 2.
5. Dilute the secondary antibody in TBST according to the manufacturer´s specification. Incubate the membrane in a clean tray containing 50 mL of diluted secondary antibody for 1 hour at room temperature on an orbital shaker.
6. Wash the membrane three times as in step 2.
7. Colour development requires the use of a commercially available alkaline phosphatase conjugate substrate kit (for this example). Follow the manufacturer´s instructions.
8. After the bands become clearly visible, stop the colour by placing the membrane in a tray containing dH_2O for at least ten minutes.