Western Blotting

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Abstract

Citation: Yongbo Cui Western Blotting. **protocols.io**
dx.doi.org/10.17504/protocols.io.h8hb9t6
Published: 18 Jun 2017

Protocol

Sample preparation: Preparation of lysate from tissues

Step 1.

1. Dissect the tissue of interest with clean tools, on ice preferably, and as quickly as possible to prevent degradation by proteases.
2. Place the tissue in round-bottom microcentrifuge tubes or Eppendorf tubes and immerse in liquid nitrogen to snap freeze. Store samples at -80°C for later use or keep on ice for immediate homogenization. For a 5 mg piece of tissue, add 300 µL of ice cold lysis buffer rapidly to the tube, homogenize with an electric homogenizer, rinse the blade twice with another 2 x 200 µL lysis buffer, then maintain constant agitation for 2 h at 4°C (eg place on an orbital shaker in the fridge). Volumes of lysis buffer must be determined in relation to the amount of tissue present; protein extract should not be too dilute to avoid loss of protein and large volumes of samples to be loaded onto gels. The minimum concentration is 0.1 mg/mL, optimal concentration is 1–5 mg/mL.
3. Centrifuge for 20 min at 12,000 rpm at 4°C in a microcentrifuge. Gently remove the tubes from the centrifuge and place on ice, aspirate the supernatant and place in a fresh tube kept on ice; discard the pellet.
4. Remove a small volume of lysate to perform a protein quantification. Determine the protein concentration by BCA or Bradford method (follow manufacturer’s instructions.).
5. Determine how much protein to load and add an equal volume 2X Laemmli sample buffer.
6. To reduce and denature your samples, boil each cell lysate in sample buffer at 100°C for 5 min. Lysates can be aliquoted and stored at -20°C for future use.

SDS-PAGE Gel Electrophoresis

Step 2.

1. Load equal amounts of protein into the wells of the SDS-PAGE gel, along with molecular weight marker. Load 20–30 µg of total protein from cell lysate or tissue homogenate, or 10–100 ng of purified protein.
2. Run the gel for 1–2 h at 100 V. The gel percentage required is dependent on the size of your protein of interest. The time and voltage may require optimization.

Transferring the protein from the gel to the membrane

Step 3.

1. The membrane can be either nitrocellulose or PVDF. Activate PVDF with methanol for 1 min and rinse with transfer buffer before preparing the stack. The time and voltage of transfer may require some optimization.
2. We are maily referred from "Western Blotting Protocol (Immunoblotting Protocol)"from Sigma-Aldrich which could be seen at "http://www.sigmaaldrich.com/china-mainland/zh/technical-documents/protocols/biology/western-blotting.html"

Antibody staining (Immunodetection)

Step 4.

1) Block the membrane for 1 h at room temperature or overnight at 4°C using blocking buffer.
2) Incubate the membrane with appropriate dilutions of primary antibody in blocking buffer. We recommend overnight incubation at 4°C; other conditions can be optimized.
3) Wash the membrane in three washes of TBST, 5 min each.
4) Incubate the membrane with the recommended dilution of conjugated secondary antibody in blocking buffer at room temperature for 1 h.
5) Wash the membrane in three washes of TBST, 5 min each.
6) For signal development, follow the kit manufacturer’s recommendations. Remove excess reagent and cover the membrane in transparent plastic wrap.
7) Acquire image using darkroom development techniques for chemiluminescence, or normal image scanning methods for colorimetric detection.