Western Blotting Protocol

Statement: The Western Blotting protocol provided here is intended for reference only. It may not be suitable for special proteins such as membrane proteins, phosphorylated proteins, high-molecular-weight proteins, low-molecular-weight proteins, or other special types of proteins. Please design a more appropriate experimental protocol based on actual conditions.

Sample preparation

1. Collect cells or tissue samples.
2. Lyse the samples using an appropriate lysis buffer (include protease and phosphatase inhibitors if necessary).
3. Centrifuge lysates at 16,000 × g for 20 min at 4°C. Collect supernatant.
4. Quantify protein concentration.
5. Mix samples with loading buffer and boil at 95-100°C for 5 min.

Note: Operate on ice as much as possible. For long-term storage, aliquot samples and preserve at -80°C freezer. Avoid freeze-thaw cycles to prevent protein degradation.

SDS-PAGE

6. Prepare SDS-PAGE gel according to manufacturer's instructions.
7. Load 20-50 μg protein to each well and molecular weight marker onto the gel.
8. Run the gel in running buffer at a constant voltage (typically around 100V) until the dye front reaches the bottom of the gel.
9.

Protein Transfer (Wet Transfer)

10. Equilibrate gel and PVDF/nitrocellulose membrane in transfer buffer for 15 min.

Note:If the NC film is selected, it needs to be treated with Western Transfer Buffer before use; If the PVDF membrane is used, it should be soaked in methanol for 5-10 min before use, and then soaked in the Western Transfer Buffer.

11. Assemble transfer sandwich (cathode-sponge-filter paper-gel-membrane-filter paper-sponge-anode). Set up the transfer apparatus following the manufacturer’s guidelines, ensuring the correct orientation of the gel, membrane, and electrodes.

Note: Avoid bubbles during transfer to ensure even protein migration.

12. Perform the transfer typically at 100V for 1 hour at 4°C.

Blocking

13. Incubate membrane in 5% non-fat milk or BSA in TBST for 1 hour at room temperature or overnight at 4°C with gentle shaking to prevent non-specific binding.
14. Wash the membrane 3 times with TBST for 5 min each to remove blocking buffer.

Primary Antibody Incubation

15. Dilute primary antibody in blocking buffer (refer to antibody datasheet for dilution).
16. Incubate membrane with antibody overnight at 4°C (or 2 hours at room temperature) on a rocking platform.
17. Wash the membrane 3 times with TBST for 5 min each to remove unbound antibody.

Secondary Antibody Incubation

18. Dilute HRP-conjugated secondary antibody in blocking buffer (refer to antibody datasheet for dilution), incubate membrane for 1 hour at room temperature with shaking

Note: If the primary antibody is conjugated with HRP, this step should be omitted and proceed directly to step 19.

19. Wash the membrane 3 times with TBST for 5 min each to remove unbound antibody.

Detection

20. Prepare chemiluminescent substrate according to manufacturer's instructions.
21. Apply substrate evenly onto membrane and capture chemiluminescence signals using a gel imaging system.

Membrane Stripping (Optional)

22. Incubate membrane in stripping buffer (e.g., 0.2 M NaOH) for 10-15 min.
23. Wash thoroughly with TBST and re-block for subsequent probing.