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WESTERN BLOTTING PROTOCOL

This protocol is a recommendation only. Please optimize the procedure since experimental conditions can vary for different tissue samples.

SAMPLE PREPARATION

1. Lyse sample in the appropriate lysis buffer.
2. Reduce and denature the sample by adding sample buffer and heat for 5 minutes at 95°C.
3. Centrifuge at 16000 x g in a microcentrifuge for 5 minutes.

ELECTROPHORESIS

4. Load 20-30ug of lysate per lane (or 100ng of purified protein) along with a molecular weight marker.
5. Prepare the running buffer and assemble the gel in the tank.
6. Run SDS-PAGE for 30 minutes at an initial voltage of 75V for 20 minutes, then 115V for 60 minutes.

TRANSFER

7. Prepare transfer buffer.
8. Cut a piece of membrane and wet in methanol. Transfer the membrane to 1x transfer buffer.
9. Assemble transfer stack.
10. Run transfer, 100V for 70 minutes.
11. Check the transfer with Ponceau or Coomassie stain.

IMMUNOBLOTTING

12. Block the membrane by incubating for 1 hour in blocking buffer (TBST with 5% BSA or milk).
13. Incubate the membrane with primary antibody in blocking buffer overnight at 4°C while gently agitating.
14. Wash 3 times in TBST while gently agitating for 10 minutes per wash.
15. Incubate the membrane with secondary antibody in blocking buffer for 1-2 hours at RT while gently agitating.
16. Wash 3 times in TBST while gently agitating for 10 minutes per wash.

DETECTION

17. Incubate the membrane at RT for 1 minute in mixture (1:1) of two ECL solutions.
18. Remove the excess liquid and wrap the membrane in transparent plastic wrap.
19. Expose to film and develop the image.