Wester Blot:

The immunoblotting technique provides information about the presence, molecular weight, and/or quantity of an antigen by combining protein separation via gel electrophoresis with specific recognition of antigens by antibodies. Immunoblotting is useful when the antigen of interest is insoluble or readily degraded and cannot be easily immunoprecipitated. Since most gel electrophoresis procedures result in denaturation of the antigen, only polyclonal and monoclonal antibodies that recognize the denatured form of an antigen can be utilized in immunoblotting. To study proteins that are expressed at very low levels, it is recommended that immunoprecipitation be followed by immunoblotting for more sensitive detection.

Materials :

- Cell lysates or other source of protein to be tested
- Immunoblotting membrane, nitrocellulose or PVDF
- Primary antibody
- Secondary antibody conjugated to HorseRadish Peroxidase (HRP) - If using mouse, rabbit, goat or sheep IgG primary antibody is recommended.
- SuperSignal™ CL-HRP Substrate System (Pierce)
- Hyperfilm™-ECL films (Amersham)

Buffers:

- Amido Black or Ponceau S Solution (Sigma Cat. No. P-7170)
- Blocking Buffer
- TBST
- Antibody Binding Buffer

Instruments:

- Mini-gel electrophoresis system and a transfer system
- Film processing room

Method:

1. Prepare samples/controls and separate proteins using an SDS-PAGE gel.
2. Transfer proteins from the gel onto Immobilon-P membranes following instructions provided by the transfer system manufacturer for best protein transfer results.
3. Stain the blot with Amido Black for one minute. Then, transfer the membrane to a dish containing the Amido Black Destaining Buffer and rock for 15-20 minutes. Examine the blot for the complete protein transfer. *Note: This step is incorporated to ascertain efficient protein transfer from the gel to the membrane. Amido Black binds to all proteins on the membrane and does not interfere with the subsequent antibody reaction with the specific protein. After destaining, look for the complete transfer of your protein marker and your test lanes.*

Alternatively, after the transfer, the gel can be stained with Coomassie blue stain to make sure that the transfer to the membrane has been completed.

4. After destaining the blot, immediately place it into the blocking buffer and rock for one hour.
5. Incubate the blot with primary antibody in the Antibody Binding Buffer overnight at 4°C. Prepare the primary antibody as recommended by the supplier or use a standard concentration of 1-2µg/ml. If using hybridoma tissue culture supernatant or serum for immunoblotting, it should be first evaluated whether the supernatant or serum can be used as "neat" or needs to be diluted 1:2, 1:5 or further for best results.
6. After the overnight incubation of the membrane with the primary antibody, wash the blot five times in TBST, each wash for 5 minutes.
7. Incubate the blot with the secondary antibody conjugated to HRP for one hour at room temperature. Prepare the secondary antibody as recommended by the supplier in the Antibody Binding Buffer.
8. After incubation of the secondary antibody, wash the blot five times in TBST, each wash for 5 minutes.
9. Develop the blot following the Pierce Chemiluminescence HRP substrate instruction.
10. Expose the blot to X-ray film for the appropriate time period. For best results, expose for ten seconds, one minute, five minutes and 20 minutes to visualize the chemiluminescence signal corresponding to the specific antibody-antigen reaction.

Buffers:

- TBS/Tween, TBST:
    - 25 mM Tris-HCl, pH 8.0
    - 125 mm NaCl
    - 0.1% Tween 20
- Blocking Buffer:
    - 5% Carnation nonfat dry milk in TBST
- Amido Black Destaining Buffer:
    - 10% methanol
    - 10% acetic acid
- Antibody Binding Buffer:
    - 1% Carnation nonfat dry milk in TBST