Oncogene Science™

IMMUNOBLOTTING PROCEDURE

INTRODUCTION:

OSI® Monoclonal Antibodies and Affinity-Purified Polyclonal Antibodies can be used to Western immunoblot proteins previously separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred onto nitrocellulose membranes. The protein antigens are reacted with antibodies and visualized using a variety of detection methods including radioactive and enzyme-labeled antibodies, protein A, and streptavidin.

MATERIALS:

1. Equipment

   - **Electrophoresis apparatus**
   - **Semidry Electroblotting apparatus**
   - **Rocker platform (i.e., Bellco)**

2. Solutions and Reagents

   - **OSI® Monoclonal Antibody Immunoblotting System containing:**

     - OSI® Monoclonal Antibody
     - Goat Anti-Mouse (or Anti-Rat) IgG Alkaline Phosphatase Conjugate
     - Two component substrate

     OR

   - **OSI® Polyclonal Antibody Immunoblotting System containing:**

     - OSI® Affinity-Purified Polyclonal Antibody
     - Goat Anti-Rabbit IgG Alkaline Phosphatase Conjugate
     - Two component substrate

   - **Tris buffered saline and Tween 20 (TBST):**

     10 mM Tris-HCl pH 8.0
     150 mM NaCl
     0.05% Tween-20

   - **TBST containing 0.5% nonfat powdered milk.** Filter before use and do not keep for longer than 1 week.

   - **Alkaline phosphatase substrate solution:**

     100 mM Tris-HCl, pH 9.5
     100 mM NaCl
     5 mM $MgCl_2$

     - For each ml of alkaline phosphatase substrate solution combine 1 ml substrate buffer with 4 µl substrate component A (Nitroblue Tetrazolium) and mix. Add 4 µl substrate component B (5-Bromo-4-Chloro-3-Indolyl- Phosphate), mix again, and use within 30 minutes

   - **Stop solution:**

     20 mM Tris-HCl pH 8.0
     5 mM EDTA

PROCEDURE:

- Electrophorese protein samples and prestained molecular weight markers.
- Transfer protein samples from polyacrylamide gel onto nitrocellulose membrane using an electroblotting apparatus.
- Block the nitrocellulose membrane for 30 to 60 minutes with TBST 0.5% milk solution at room temperature using about 1 ml per cm^2 membrane.
- Wash the nitrocellulose membrane by soaking in TBST for 10 minutes.
- Repeat the wash step.
- Incubate the nitrocellulose membrane for 60 minutes with primary antibody (about 10 µg/ml diluted in TBST, 0.5% milk).
- Wash the nitrocellulose membrane by soaking in TBST for 10 minutes.
- Repeat the wash step.
- Incubate with second antibody enzyme conjugate (0.2 µg/ml diluted in TBST) for 60 minutes.
- Wash the nitrocellulose membrane by soaking in TBST for 10 minutes.
- Repeat wash step twice.
- Develop the blot with alkaline phosphatase substrate solution.
- As soon as the bands develop to the desired intensity, stop the reaction by washing the blot in stop solution.
- Dry the blot in air at room temperature.
- Photograph and/or mount the blot under a plastic sheet in a laboratory notebook.

NOTES:

- The sensitivity of immunoblotting is dependent on the efficiency of transfer from the polyacrylamide gel onto the nitrocellulose membrane. In general thinner and lower percentage gels give greater transfer efficiency and thus higher immunoblotting sensitivity. The efficiency of transfer can be checked by staining (i.e., Coomassie) the gel following electrophoretic transfer.
- Nonfat powdered milk is an excellent blocking agent, but other proteins (such as casein, gelatin, bovine serum albumin, ovalbumin or hemoglobin) also are used. Blots should be rocked during blocking and subsequent steps. Strips of blots corresponding to individual gel lanes can be cut with a razor blade or scalpel and reacted individually in specially divided trays or tubes.
- Any wash step may be extended to overnight at 4°C, if desired for convenience.
- The concentrations of both primary and secondary antibodies should be titrated for optimum sensitivity and lowest background.
- The second antibody alkaline phosphatase conjugate and substrate provided are recommended, but other substrates and enzymes also may be used.
- The second antibody alkaline phosphatase conjugate may be used for other applications such as ELISA but the appropriate substrate must be used i.e., one yielding a soluble product.
- Some fading of blots may occur with time and fading may be accelerated by exposure to direct light.
- Rewetting faded blots will make the bands appear more intense.
- If the substrate solutions develop precipitates during storage at 4°C or -20°C, warm them to room temperature and mix. A sonicating water bath also may be used. A small amount of precipitate in these solutions will not harm the performance of the product. Keep substrate solutions away from open flames and avoid contact with skin, eyes and mouth.
- Improved performance may be obtained using subcellular fractions for immunoblotting, i.e., nuclei for nuclear proteins or membranes for membrane receptors. Concentration of antigens by immunoaffinity chromatography or immunoprecipitation helps to obtain more intense bands on immunoblots.

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ORDERING INFORMATION
Oncogene Science, Inc. research reagents may be ordered by mail at the address shown at left, by telephone or by fax. Terms are net 30 days F.O.B. Uniondale, NY