PROMETHEUS®
Protein Biology Products

Instruction Manual

Polyvinylidene Fluoride (PVDF) Membranes
Catalog No: 83-646R, 84-895

Western Transfer: Blotting and Detection of Proteins

1. Sample Fractionation
   Fractionate purified protein sample in standard SDS-PAGE or IEF gel. If the effectiveness of the transfer will be evaluated, then run a duplicate sample in an outer lane, as well as a prestained molecular weight marker.

2. PVDF Membrane Preparation
   For membrane preparation, float the PVDF membrane, cut to the size of the gel, in 100% methanol until completely saturated (not more than 15 seconds). Briefly, rinse the membrane in deionized water and then equilibrate the membrane in Western transfer buffer for several minutes.

   Note: Complete wetting of the membrane is important to ensure proper protein binding. Abrupt wetting can lead to entrapment of air bubbles in the membrane, which can block transfer of proteins.

   Note: PVDF is a hydrophobic membrane and for aqueous based applications, it needs to be prewetted in methanol.

2. Transfer
   Transfer according to the electroblotting apparatus instructions. Transfer in 25mM Tris-HCl/192mM glycine/20% methanol, pH 8.3, or another suitable buffer.

   Note: If there is a sidedness to the membrane, place the smooth side of the membrane next to the gel for the transfer.

4. Transfer Determination
   To check for transfer of the sample onto the membrane, cut the duplicate sample lane from the blot and stain using either Amido black or Ponceau S red as described below.
   A. Amido black: incubate blot in a solution of 0.1% amido black in 25% isopropanol/10% acetic acid and destain in the same buffer without the amido black. A slight shrinking of the membrane may occur with this method.

B. Ponceau S red: stain blot in 0.5% Ponceau S red in 1% acetic acid for 2-5 minutes. Rinse membrane strip with water to destain. Washing with water will eventually remove the stain completely. For a permanent stain, destain in 10% isopropyl alcohol/10% acetic acid.

To determine if the protein has eluted from the gel, stain gel with Coomassie blue or with a silver stain.

5. Blocking of Membrane
   After transfer, wash membrane in 1X Tris buffered saline pH 7.4 (TBS) for 5 minutes. Decant the TBS and block membrane for 1 hour at room temperature in 1XTBS containing one of the blocking agents listed below. Use at least 0.3ml per cm^2 of membrane and gently rock.
   1-3% casein
   0.5-5% nonfat dry milk
   1-3% bovine serum albumin (BSA)
   1-3% gelatin

   Note: If the PVDF membrane has dried after transfer, then rewet the membrane in 100% methanol prior to the 1X TBS wash.

6. Washing
   Using 0.3ml per cm^2 of membrane, wash the blot three times with 1XTBS, 0.05% Tween 20. Each wash is 5 minutes, with gently rocking at room temperature.

7. Binding of Primary Antibody
   Dilute 0.5-10 µg/ml of primary antibody in 1XTBS, 0.05% Tween 20 solution (may add blocking agent if background is high). Use 0.3ml per cm^2 of membrane. Place washed, blocked membrane in the primary antibody solution. Rock at room temperature for 60 minutes or longer. Optimum concentrations of primary antibody should be determined empirically.

8. Washing
   Using 0.3ml per cm^2 of membrane, wash the blot three times with 1XTBS, 0.05% Tween 20. Each wash is 5 minutes, with gently rocking at room temperature.

9. Addition of Secondary Antibody Conjugate
   Dilute the appropriate alkaline phosphatase or horseradish peroxidase (HRP) secondary conjugate in 1XTBS, 0.05% Tween 20 solution. Use 0.3ml per cm^2 of membrane. Gently rock the blot for 60 minutes at room temperature.

10. Washing
    Using 0.3ml per cm^2 of membrane, wash the blot three to five times with 1XTBS, 0.05% Tween 20. Each wash is 5 minutes, with gently rocking at room temperature.

11. Non-isotopic Detection

    For alkaline phosphatase secondary antibody conjugate, colorimetrically detect blots using BCIP/NBT* or TMB** –blotting substrate. Follow the manufacturer's instructions. A digital image of the blot is taken and data analysis is performed.

    For chemiluminescent detection of blots use a luminol-based substrate, such as SuperSignal ™ West Dura, in conjunction with an HRP-secondary antibody conjugate. Follow manufacturer's instruction. Signal is captured using a CCD imager or X-ray film and data analysis is performed.

    ***BCIP/NBT is 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium**
    ****TMB is 3,3',5,5'-Tetramethylbenzidine**