Performance Study of KPL's Membrane Substrates on Transfer Membranes

Purpose: To study the performance of KPL's Membrane Substrates, BCIP/NBT Phosphatase Substrate System (Cat. No. 50-81-00), 4 CN Peroxidase Substrate System (Cat. No. 50-73-00), and TMB Membrane Peroxidase Substrate System (Cat. No. 50-77-00), on a number of commercially available transfer membranes.

Materials: The following membranes were used in this study.

1. Biodyne A (Pall)
2. Biodyne B (Pall)
3. PVM Membrane (Pall)
4. Immunodyne (Pall)
5. Nytran Transfer Media (S&S)
6. Nylon 66 (S&S)
7. Nitro Plus 2000 (MSI)
8. MagnaGraph Nylon 66 (MSI)
9. Immobilon-P (Millipore)
10. Nitrocellulose (S&S) - Control Membrane

Test Parameters: A dot ELISA procedure was used to evaluate the performance of the membrane substrates and the various transfer membranes. Although each membrane manufacturer suggests certain assay procedures, a standardized assay was developed for the purpose of this study. The assay is as follows:

1. Set up dilution plate by performing 12 two-fold dilutions across a single row of a microtiter plate with Mouse IgG (Cappel), starting at a 0.1mg/ml concentration in PBS.
2. Wet membranes according to manufacturer recommendations. Note: Pre-wet all membranes in reagent quality water except for Immobilon-P (9) which requires wetting with 100% methanol for 2-5 seconds. Remove excess methanol by rinsing the membrane in water.
3. From each well in the dilution plate, transfer 1.0 µl of the diluted Mouse IgG to gridded membrane strips using a microdispenser. Allow strips to incubate for approximately 5 minutes for protein to adhere to the membrane.
4. Block strips with 5% BSA for 1 hour at room temperature.
5. Incubate strips with Peroxidase or Phosphatase-labeled Goat anti-Mouse IgG (H+L) (Catalog No. 04-18-06 and 05-18-06) at a concentration of 0.1 µg/ml in BSA Diluent /Blocking Solution (Cat. No. 50-61-00) for 1 hour at room temperature.
6. Wash strips 3 times with 3 minutes soak periods using Wash Solution (Cat. No. 50-63-00). After final wash, rinse strips with water.
7. Add substrate (BCIP/NBT, 4 CN, or TMB Membrane Substrate). The reaction time for each substrate is different. The substrate reactions are monitored and stopped before background staining become too high.
8. Stop substrate reaction by rinsing in water for 10-20 seconds.
9. Allow strips to air dry before storing.

Results: In this study, color reactions were observed on all membranes using each of the three substrate systems. However, differences in membrane sensitivity and non-specific background staining were observed.

Membrane Sensitivity: When using 4 CN Peroxidase Substrate, all membranes appeared approximately equal in sensitivity. When using the BCIP/NBT Phosphatase Substrate and TMB Membrane Peroxidase Substrate, membranes 1,2, and 4-8 appeared equal in sensitivity to the control membrane, nitrocellulose. Immobilon-P (9) and the PVM Membrane (3) are approximately 2 two-fold dilutions of the antigen more sensitive than nitrocellulose.

Membrane Background: The 4 CN Peroxidase Substrate System exhibited negligible levels of background staining on all membranes tested. The use of TMB Membrane Peroxidase Substrate and BCIP/NBT Phosphatase Substrate resulted in higher levels of background color on all membranes except nitrocellulose (10) and Nitro Plus 2000 (7). The highest levels of background were seen with the nylon based membranes (1-6,8). The manufacturers recommendation of 10% BSA blocking at 37°C overnight did not seem to reduce the levels of background seen on the nylon- based membranes. Immobilon-P (9) showed intermediate levels of background color. The background on all membranes could be effectively reduced by decreasing incubation time with the substrate.

Conclusions: In this study the three membrane substrates tested, BCIP/NBT Phosphatase Substrate System, 4 CN Peroxidase Substrate System, and TMB Membrane Peroxidase Substrate System, produced color reactions that were stopped effectively with water and did not fade significantly over time. The membranes showed different levels of sensitivity with Immobilon-P and PVM Membranes proving to be slightly more sensitive, regardless of the substrate used. Background staining when using BCIP/NBT Phosphatase Substrate and TMB Membrane Substrate was highest with the nylon membranes (1-6,8). Modifications to the above assay procedure, as suggested by the membrane manufacturers, may help in reducing the levels of background staining observed.

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