DOT BLOT PROTEIN

Dot blotting is a method of applying proteins directly onto a membrane. A dissolved sample is pulled through the membrane by either applying a vacuum, absorption or intrusion; proteins bind to the membrane and the other sample components pass through. The proteins on the membrane are then available for analysis. This technique can be used either as a qualitative method for rapid screening of a large number of samples or as a quantitative technique. It is especially useful for testing the suitability of experimental design parameters.

When preparing blots by filtration, keep in mind the following:
• Detergents can inhibit the binding of proteins to the membrane. Buffers used for sample dissolution and washing should contain no more than 0.05% detergent, if required. **For Sample Buffer (0.4%SDS) dilute 1:7 with buffer to achieve 0.05% SDS.**
• Choose a sample volume large enough to cover the exposed membrane in each well, but be careful not to exceed the binding capacity of the membrane. In a blotting unit, the binding capacity of Immobilon-P is typically 5 to 10 mg/well, depending on the particular protein, the surface area of exposed membrane, the filtration rate, and the buffer formulation.
• High particulate loads or viscosity will reduce the flow rate and clog the membrane. Centrifuge samples with particulates, and apply only the supernatant to the membrane. Dilute viscous samples in buffer.

Manual Spotting Methods

Required Equipment and Solutions

• Two sheets of Immobilon-P membrane, cut to size for blotting unit*
• Filter paper, cut to size for blotting unit (i.e. Whatman™ 3MM filter paper)
• Methanol, 100%
• Ultrapure water (produced by the Milli-Q® System)
• Buffer, for sample loading and wash
• Blotting unit, dot blot or slot blot format

Set Up

1. Prepare Immobilon-P membrane:
   a. Wet the membrane by laying it on the surface of methanol for 15 seconds. Do not immerse. The membrane should uniformly change from opaque to semi-transparent.
   b. Carefully place the membrane in ultrapure water and soak for 2 minutes.
   c. Carefully place the membrane in buffer and let equilibrate for at least 5 minutes.
2. Dissolve the sample in buffer.
   a. If the sample solution is cloudy, centrifuge to remove particles.
   b. If the sample is viscous, dilute with additional buffer.

Stack

3. Assemble stack as follows (from the bottom up):
   Place paper towels on work surface (bottom towels should remain dry throughout blotting procedure).
   Place dry filter paper (i.e. Whatman 3MM paper) on paper towels.
   Place filter paper (prewet with buffer) on dry filter paper.
   Place prewet Immobilon-P membrane on wet filter paper.
4. Spot 1 – 5 mL of sample onto membrane. Sample should wick into membrane. Membrane should be wet enough to absorb sample, but not so wet that sample spreads across membrane.
5. After sample is absorbed, place membrane on clean filter paper to dry.
References: