Minifold I 96-Well System

Quick Reference Protocol Card

Codes: 10447850 Minifold I Spot-Blot System
10447900 Minifold I Dot-Blot System
10447941 Minifold I Slot-Blot System

Introduction

The Minifold I manifold is a 96-well filtration/incubation unit for processing dot-blot assays of nucleic acids or proteins. The unique design of the device eliminates cross-lateral flow of reagents across the solid phase, and forms discrete, uniform test dots for easy isolation and quantitation of results. The Minifold I system (Photo 1) includes (1) a sample well plate with clamping plate and silicone O-rings; (2) a filtration plate and (3) a vacuum plenum. The Minifold is easily adjusted for use with many different types of transfer matrices for optimal sample retention and biological activity.

The Minifold I, constructed from acrylic plastic, can be decontaminated with 10% ethanol or 10% NaOH and should not be autoclaved.

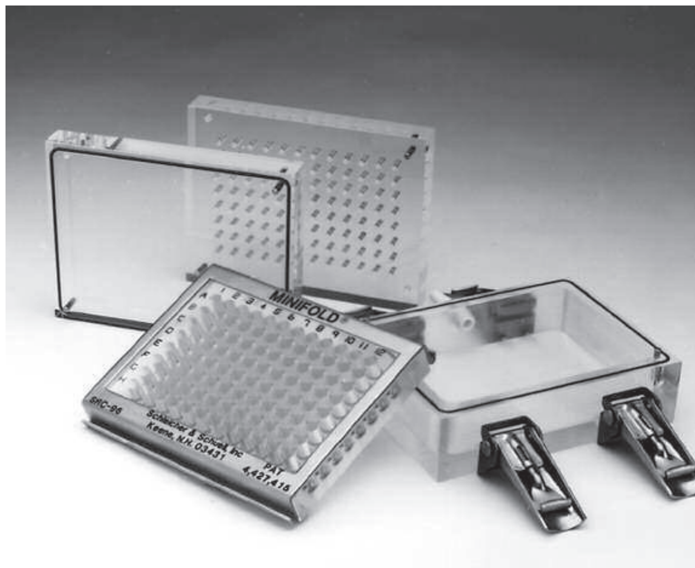

Photo 1

Filtration/Immobilization of Sample

1. Select the appropriate transfer matrix for your application. Prepare the membrane or filter as indicated in the instructions for that medium. Amersham™ Protran nitrocellulose, Amersham Protran Supported nitrocellulose or Nytran™ nylon membranes need only be saturated in buffer (e.g., SSC for nucleic acids, PBS for proteins or other appropriate solvents). Other matrices may require activation steps prior to sample immobilization.

   Back the transfer support with 1–2 sheets of prewet Whatman™ 3MM Chr blotting paper. Place the filter support plate on top of the vacuum plenum (Photo 2), aligning it with the registration pins on either corner. Align the corners of the filter sheets on the support plate; the corners of the Whatman 3MM Chr blotting paper that lie over the pins should be removed. Run a pipette over the surface of the membrane or filter and the Whatman 3MM Chr blotting paper layers to eliminate air bubbles.

   If fewer than 96 samples are to be applied, a smaller piece of filter medium can be cut. Block off the unused portion of the filter support plate with Parafilm™ to ensure maximum vacuum.

2. Place the sample well plate, with O-rings facing down, on the top of the filter. Be sure each O-ring is fully seated to prevent lateral flow. Again, registration pins ensure proper alignment. Place clamping plate on top of the sample well plate (Photo 3).

NOTE: The dot-blot sample well plate can be replaced by a 96-well spot-blot plate.

3. Clamp the "sandwich" together with the adjustable stainless steel latches. These convenient clamps are adjustable so that filter media of various thicknesses can be clamped quickly, easily and firmly. The clamping plate ensures that even pressure is applied to all sides of the plate. Close a clamp on one side, followed by the opposite diagonal clamp, until all four clamps are in place (Photo 3).

NOTE: Overtightening of the clamps may result in sample diffusion in the middle wells of the Minifold. In addition to adjusting the clamps, proper tension may be achieved by adding or removing 1 sheet of Whatman 3MM Chr blotting paper.

4. With the vacuum on, apply samples (200–500 µl) into the wells of the Minifold device (Photo 4). Sample wells are identified alphabetically and numerically for easy orientation in the standard microtiter format.

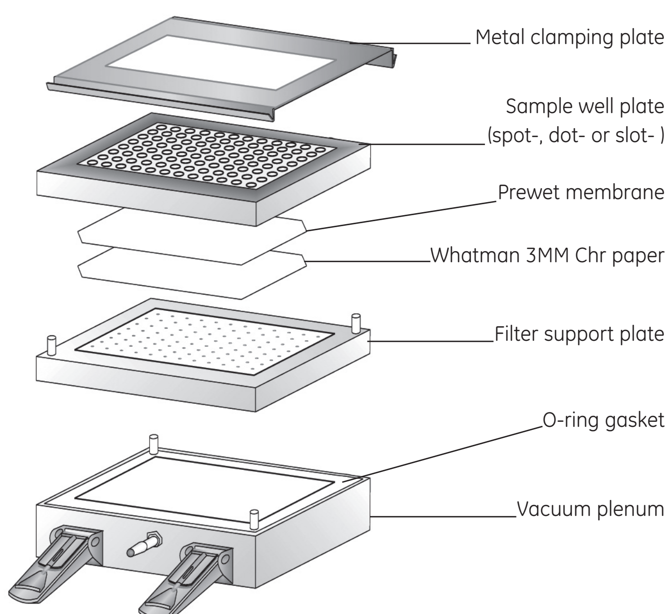

Photo 2

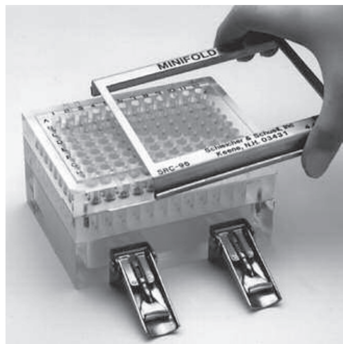

Photo 3

5. After completing filtration steps, unclamp the Minifold and remove the sample well plate. Remove the membrane or filter and any underlying filter paper with forceps (Photo 5).
6. Follow the blocking and detection procedures indicated for the particular membrane.

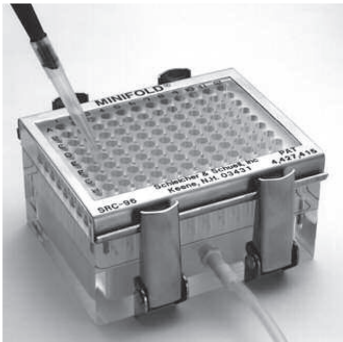

Photo 4

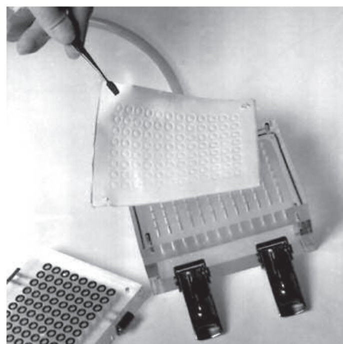

Photo 5

Cleaning and Decontamination

To clean the Minifold I unit, wash thoroughly in any general laboratory-grade detergent and rinse briefly in 10% ethanol. NEVER soak the Minifold I apparatus, as warping of the plates may occur. Radioactive substances should be removed with an isotope-removing cleaner. To make the unit RNase-free, wash in 10% NaOH for 5 min, then rinse thoroughly in RNase-free deionized water. Do not treat the unit with diethylpyrocarbonate (DEPC). The acrylic Minifold I unit should not be autoclaved

References

1. Kafatos, F. C. *et al.*. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucl. Acids Res. **7**:1541-1552 (1979).
2. Dobner, P.R. *et al*. Thyroid or glucocorticoid hormone induces pre-growth-hormone mRNA and its probable nuclear precursor in rat pituitary cells Proc. Natl. Acad. Sci. **78**:2230-2234 (1981).
3. Hawkes, R., E. *et al*. Anal. Biochem. Identification of concanavalin A-binding proteins after sodium dodecyl sulfate-gel electrophoresis and protein blotting. **119**:142-147 (1982).
4. Bonfanti, M. *et al*. BioTechniques. O6-methylguanine inhibits the binding of transcription factors to DNA.**19**:5739-5742 (1991).

Ordering Information

Description	Product No.
Minifold I Spot-Blot System	10447850
Minifold I Dot-Blot System	10447900
Minifold I Slot-Blot System	10447941
Protran BA83 102 × 133 mm 10/PK	10402488
Protran BA85 102 × 133 mm 10/PK	10402588
Replacement O-rings 50/PK	10447902

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