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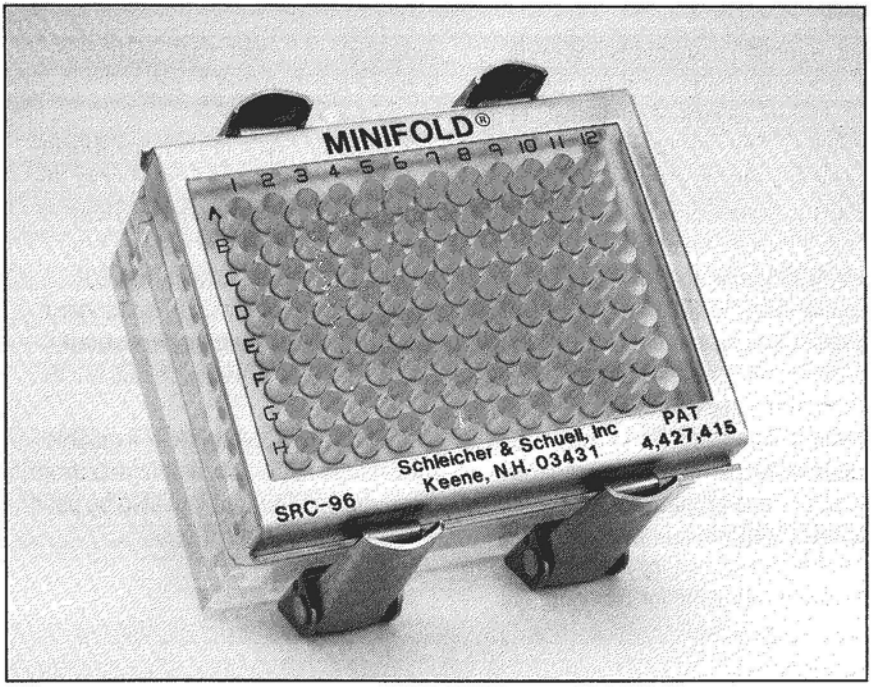
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S&S Minifold® I

General Instructions for Use

Schleicher & Schuell

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The S&S Minifold® I Filtration Manifold for Dot-Blot Assays

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. An accessory incubation plate (4) is also available for carry-ing out individual assays on each of the 96 wells of the manifold.

The Minifold I manifold is available in either a standard acrylic unit or in the durable, chemically resistant Delrin® model. The Delrin model is recommended for sterile procedure as it withstands autoclaving; the standard acrylic Minifold I can be decontaminated with 10% ethanol or 10% NaOH.

The S&S cutting template is available for rapid isolation of sample dots from filter sheets. The unit can also be used with individual filter discs.

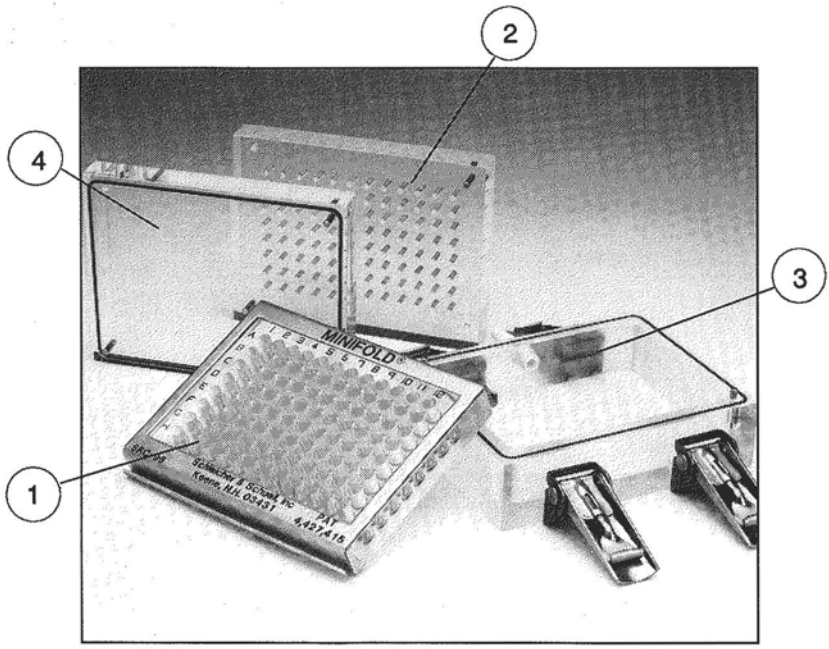


Photo 1

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Filtration/Immobilization of Sample

1. Select the appropriate S&S transfer matrix for your application. Prepare the membrane or filter as indicated in the instructions for that medium. S&S NC™ 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 (e.g., DEAE anion-exchange membranes) may require activation steps prior to sample immobilization.

   Back the transfer support with 1-2 sheets of pre-wet S&S GB002 gel blot 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 GB002 paper that lie over the pins should be removed. Run a pipet over the surface of the membrane or filter and the GB002 layers to eliminate air bubbles.

   It may be advisable to use individual discs (see step 2) when using glass fiber filter papers since the thickness of the medium may result in unacceptable lateral flow.

   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).

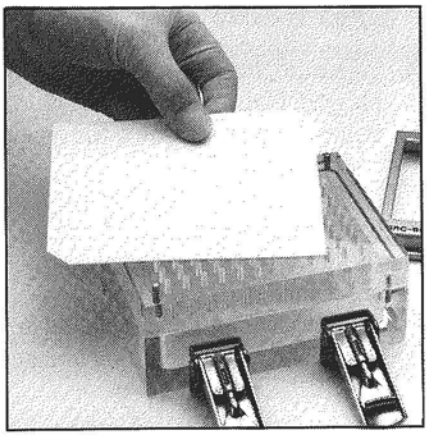


Photo 2

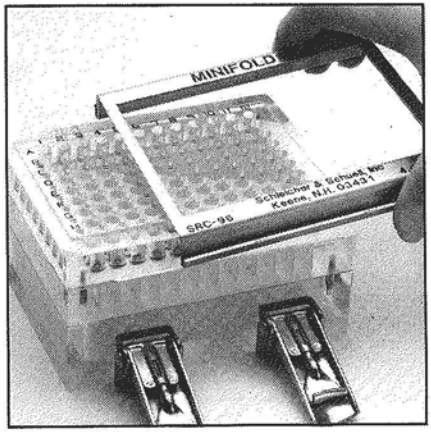


Photo 3

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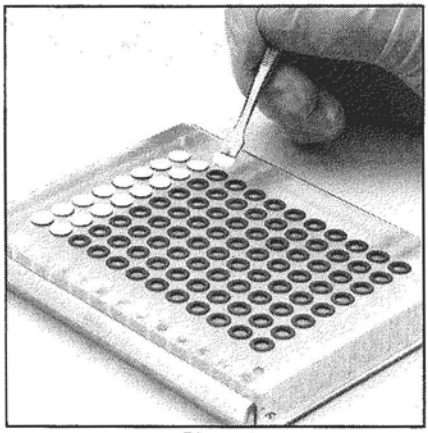


Photo 4

S&S filter media are also avail-abe in 7.0 mm discs for carrying out filtration on a few samples at a time. Using flat-blade forceps, place filter discs on the O-rings of the sample well plate (photo 4). Slight pressure on the filter will cause it to adhere to the O-ring. Moistened filters may be easier to handle. Place the filter support plate on the base of the sample well plate (aligning registration pins) and carefully invert (photo 5). Place the two plates on the vacuum plenum

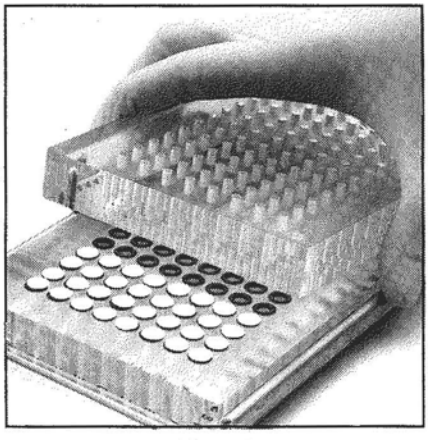


Photo 5

followed by the clamping plate on top of the sample well 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 6).

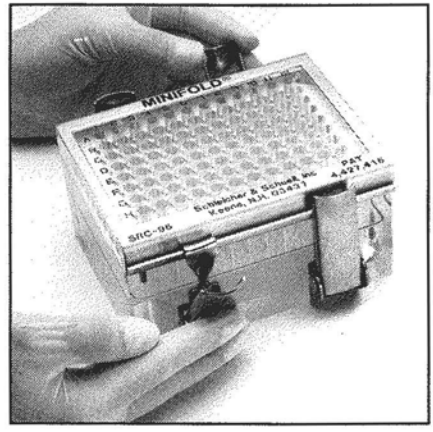


Photo 6

4. To ensure that no wells are plugged, filter a small amount of buffer through each well, and apply vacuum (photo 7).

Sample can now be introduced into the Minifold device. Sample wells are identified alphabetically and numerically for easy orientation in the standard microtitration format.

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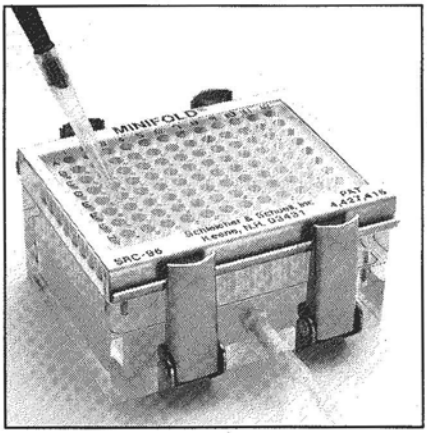


Photo 7

5. If successive washes of specified volumes are required, the surface of the sample well plate can be flooded — with the vacuum off — to fill each well. With the vacuum on, a wash volume of 500 µl is optimal.

Incubation in Individual Wells

6. 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. At this point the filter or membrane with the immobilized sample may be incubated in the Minifold device with the incubation plate, or processed as an entire sheet for other applications.

   To carry out incubations in individual wells, remove the filter support plate and insert the incubation plate, O-ring side-up (photo 8). Orient the membrane or paper and the filter paper over the alignment pins. Place sample well plate and clamping plate on top of the "sandwich" and clamp evenly. Place sample in the individual wells and incubate as required.

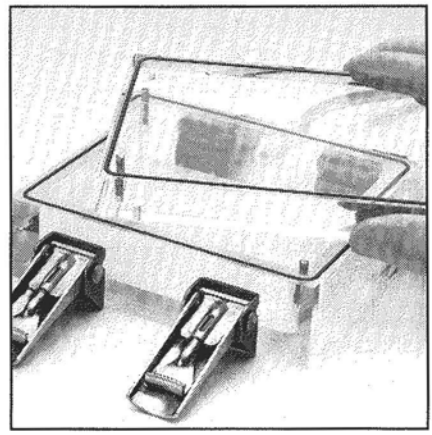


Photo 8

   If using individual discs instead of sheets, invert entire apparatus, loosen clamps and remove plenum and incubation plate gently, so individual discs are not disturbed. Replace incubation plate and vacuum plenum on top of the sample well plate and re-invert apparatus.

Reagent Dispensation and Washing

7. Remove contents of each well after incubation is completed and wash each well. Washes may also be carried out with the filter support plate in place instead of the incubation plate.

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8. Proceed to carry out any additional incubation or washing steps, then remove membrane or paper from the unit. The filter sheet can now be prepared for quantitation of the individual dots or other procedures. When using individual filter discs, remove incubation plate as directed for removal of the filtration plate, step 6.

Isolation of Individual Dots

9. To cut individual sections from filter sheets, the S&S Cutting Template may be used. The template's dimensions and the location of the cutting slits make perfect registration fast and easy. To use the template, place a sheet of S&S GB002 filter paper on an even surface, and place the membrane or filter sheet on top of it. Notch filter to orient it properly for easy identification of each well with the A-H/1-12 format. Align the template with the filter and cut evenly with a sharp razor blade or scalpel, pressing down firmly with the template. To cut lengthwise, orient the template with the top and sides flush with the filter; to cut along the width, align the 4" side of the filter with the indentations on the template and across the bottom edges. When reorienting template, lift carefully so as not to disturb the cut pieces (figures 1 and 2).

Cleaning and Decontamination

To clean the standard Minifold I unit, wash thoroughly in any general laboratory grade detergent, and rinse briefly in 10% ethanol (*do not soak*). Radioactive substances should be

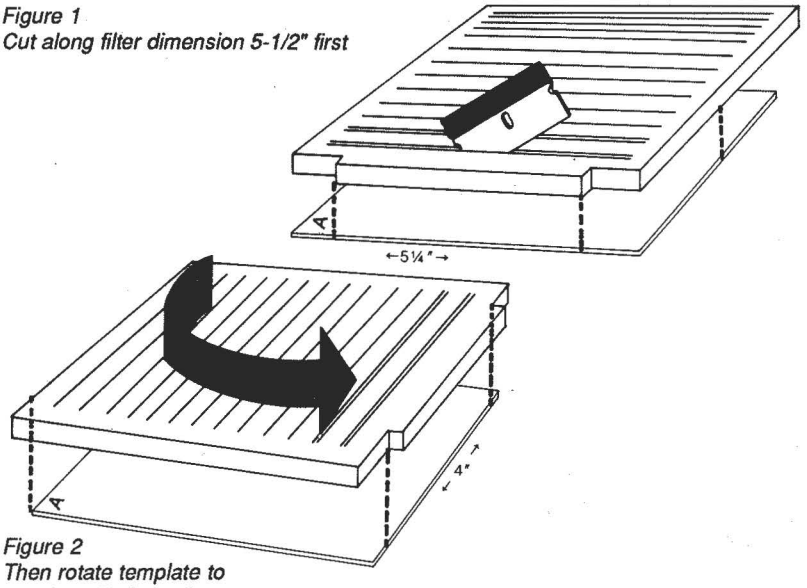


Figure 1
Cut along filter dimension 5-1/2" first

Figure 2
Then rotate template to cut short 4" dimension

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removed with an isotope-removing cleaner. To make the unit RNase -free, soak in 10% NaOH for 5 minutes, then rinse thoroughly in RNase-free deionized water. Do not treat the unit with diethylpyrocarbonate (DEPC). The standard acrylic Minifold I unit should not be autoclaved; however, the Delrin model may be sterilized by autoclaving at 121° C, 1 bar, for 15 min.

References

1. Kafatos, F.C., C.W. Jones and A. Efstratiadis. 1979. Nucl. Acids. Res. 7:1541-1552.
2. Dobner, P.R., E.S. Kawasaki, L. Yu and F.C. Bancroft. 1981. Proc. Natl. Acad. Sci. 78:2230-2234.
3. Hawkes, R., E. Niday and J. Gordon. 1982. Anal. Biochem. 119:142-147.

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Ordering Information

Description	Order #
Minifold I System (acrylic, 3 component)	27510
Minifold I System & Incubation Plate 35050 1 Manifold with vacuum tube adaptor & adjustable clamps 1 Minifold I Incubation Plate 5 S&S NC™ nitrocellulose sheets 5 Gel blot paper sheets (GB002), precut 10 Replacement O-rings	
Minifold I System (Delrin, 3 component)	27560
Minifold I Cutting Template	27520
Minifold I Incubation Plate	34960

Transfer Membranes	4" x 5-1/4"	7 mm discs
NC-BA85 (0.45 µm)	00840 (10/pk)	20230 (100/pk)
NC-BA83 (0.2 µm)	00790 (10/pk)	
APT paper	26220 (2/pk)	
Nytran (0.2 µm)	01090 (10/pk)	
Nytran (0.45 µm)	01040 (10/pk)	
NA45 (DEAE ion-exchange)	23430 (5/pk)	23260 (100/pk)
#30 glass fiber filters	06560 (50/pk)	06350 (100/pk)
GB002 paper	39690 (100/pk)	
GB003 paper	31480 (100/pk)	
#470 filter paper	05080 (100/pk)	04470 (100/pk)

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