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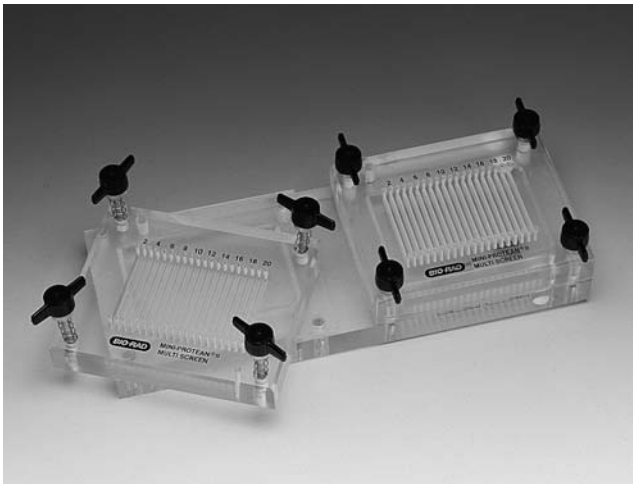
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Mini-PROTEAN® II Multiscreen Apparatus

Instruction Manual

Catalog number
170-4017

BIO-RAD

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Section 1
Introduction

With the Mini-PROTEAN II multiscreen apparatus you can quickly and easily screen up to 40 different antibody or serum samples on Western blots, without having to cut the membranes into strips. Only 600 µl of sample is used per channel, eliminating waste of precious antibody. Two individual and independent sample templates allow screening of either one or two mini blots.

The multiscreen apparatus is simple to operate. Electrophorese an antigen sample on an SDS-PAGE mini gel using the Mini-PROTEAN II cell, and blot it onto nitrocellulose or Zeta-Probe® membrane with the Mini Trans-Blot®, Trans-Blot, or Trans-Blot SD cell. After blocking the unreacted sites, clamp the membrane between the gasket and sample template. The assembly is held together with four screws, and the rubber sealing gasket prevents any well-to-well leakage. Pipet serum or antibody samples into each of the channels for incubation with the anitgen. Wash solutions can be easily introduced with the Eppendorf® Repeater™ pipet, and are rapidly removed by vacuum aspiration. The Multiscreen apparatus is compatible with all common Western blotting procedures.

1.1 Specifications

Materials	
Multiscreen apparatus	Acrylic plastic
Multiscreen gasket	Silicone rubber
Shipping weight	1.4 kg
Overall size	11 x 27 x 6 cm (W x L x H)
Membrane size	7 x 8.4 cm
Channel dimensions	2.5 mm x 5.2 cm x 5 mm (W x L x H)
Channels per sample template	20

Section 2
Equipment and Reagents

Catalog Number	Product Description
170-4017	Mini-PROTEAN II Multiscreen Apparatus, includes Multiscreen sample templates, 2 Gaskets and Base Plate
170-4018	Mini-PROTEAN II Multiscreen Gaskets, 2
Mini-Protean II Cell	
165-2940	Mini-PROTEAN II Cell, includes 10 well combs, 0.75 mm spacers (4), Electrode Core with Gaskets, lower buffer chamber, lid with cables, 3 sets Glass Plates, Clamp Assemblies (2), Casting Stand with Gaskets, leveling bubble, and instructions
Transfer Cells	
170-3930	Mini Trans-Blot Electrophoretic Transfer Cell
170-3935	Mini Trans-Blot Module
170-3946	Trans-Blot Electrophoretic Transfer Cell, with plate electrodes
170-3910	Trans-Blot Electrophoretic Transfer Cell, with standard electrodes
170-3940	Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell

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Catalog Number	Product Description
Power Supplies	
165-5052	PowerPac 200 Power Supply, 100/120 V
165-5053	PowerPac 200 Power Supply, 220/240 V
Related Instruments	
170-6545	Bio-Dot® Microfiltration Apparatus
170-6542	Bio-Dot SF Microfiltration Apparatus
Blotting Media	
Nitrocellulose Membrane (0.45 micron)	
162-0115	Nitrocellulose Membrane, roll, 33 cm x 3 m, 1
162-0113	Nitrocellulose Membrane, sheets, 20 x 20 cm, 5
162-0116	Nitrocellulose Membrane, sheets, 15 x 15 cm, 10
162-0114	Nitrocellulose Membrane, sheets, 15 x 9.2 cm, 10
162-0117	Nitrocellulose Membrane, sheets, 9 x 12 cm, 10
162-0145	Nitrocellulose Membrane, sheets, 7 x 8.4 cm, 10
Nitrocellulose Membrane (0.2 micron)	
162-0112	Nitrocellulose Membrane, roll, 33 cm x 3 cm, 1
162-0146	Nitrocellulose Membrane, sheets, 7 x 8.4 cm, 10
162-0147	Nitrocellulose Membrane, sheets, 13.5 x 16.5 cm, 10
Immun-Blot® Assay Kits	
Bio-Rad's Immun-Blot assay kits contain the necessary components and instructions for performing immune detection assays on blotted membranes.	
170-6460	Immun-Blot Assay Kit - Goat Anti-Rabbit IgG (H+L) AP Conjugate
170-6461	Immun-Blot Assay Kit - Goat Anti-Mouse IgG (H+L) AP Conjugate
170-6462	Immun-Blot Assay Kit - Goat Anti-Human IgG (H+L) AP Conjugate
170-6463	Immun-Blot Assay Kit - Goat Anti-Rabbit IgG (H+L) HRP Conjugate
170-6464	Immun-Blot Assay Kit - Goat Anti-Mouse IgG (H+L) HRP Conjugate
170-6465	Immun-Blot Assay Kit - Goat Anti-Human IgG (H+L) HRP Conjugate
170-6466	Immun-Blot Assay Kit - Protein A HRP
170-6467	Immun-Blot Assay Kit - Protein G HRP
Total Protein Detection Kits	
170-6512	Biotin-Blot Protein Detection Kit
170-6517	Enhanced Colloidal Gold Total Protein Detection Kit
Blotting Standards	
161-0305	Prestained SDS-PAGE Standards, Low Range
161-0309	Prestained SDS-PAGE Standards, High Range
161-0318	Prestained SDS-PAGE Standards, Broad Range
161-0307	Biotinylated SDS-PAGE Standards Kit, Low Range, HRP
161-0308	Biotinylated SDS-PAGE Standards Kit, Low Range, AP
161-0312	Biotinylated SDS-PAGE Standards Kit, High Range, HRP
161-0313	Biotinylated SDS-PAGE Standards Kit, High Range, AP
161-0321	Biotinylated SDS-PAGE Standards Kit, Broad Range, HRP
161-0322	Biotinylated SDS-PAGE Standards Kit, Broad Range, AP

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Section 3
Special Handling Features

The multiscreen apparatus can be cleaned with a mild, non-abrasive detergent but not be autoclaved. Do not subject the unit to temperatures greater than 50 °C, as this will warp acrylic plates. If the unit becomes warped, it will no longer provide a proper seal. Heating the apparatus to >50 °C voids all warranties.

3.1 Chemical Stability

Chemicals compatible with acrylic plastic:

hydrochloric acid	< 50% ethanol
sodium hydroxide	< 50% ethanol

Chemicals that will attack acrylic plastic:

all polar aromatic solvents or chlorinated hydrocarbons, esters, and ketones
glacial acetic acid
chromic acid
trichloroacetic acid
> 50% ethanol
> 50% methanol

Section 4
Multiscreen Operating Procedure

4.1 Preparation for the Immunoassay in the Multiscreen Apparatus

1. Electrophorese the antigen sample into a mini gel following the instructions provided with the Mini-PROTEAN II cell. The maximum length of the separating gel should not exceed 5.2 cm, the length of the channels on the multiscreen sample template. The stacking gel should be cast with the Mini-PROTEAN II preparative comb. This comb contains one large sample well and one reference well.

2. Blot the gel to nitrocellulose or Zeta-Probe membrane, using a 7 x 8.4 cm membrane size. Refer to the Mini Trans-Blot, Trans-Blot or Trans-Blot SD cell instruction manual for electrophoretic transfer procedures.

3. Following the transfer, mark the outline of the gel on the membrane using a pen or pencil. This will aid in aligning the blot with the sample template. Block the unreacted sites on the blot with a blocking solution, 1 hour at room temperature for nitrocellulose, 2 hours at room temperature for Zeta-Probe membrane. Rinse the blocked blot in TBS before applying to the multiscreen apparatus. See Section 5 for buffer formulations.

   Note: Always use forceps or wear gloves when handling membranes.

4.2 Assembly of the Multiscreen Apparatus

1. Clean and dry the multiscreen apparatus and gaskets prior to assembly.

   Note: Do not heat the apparatus to temperatures greater than 50 °C. This will cause the unit to warp.

2. Place the sealing gasket onto the base plate with the raised surface down, using the guide pins to help align the gasket.

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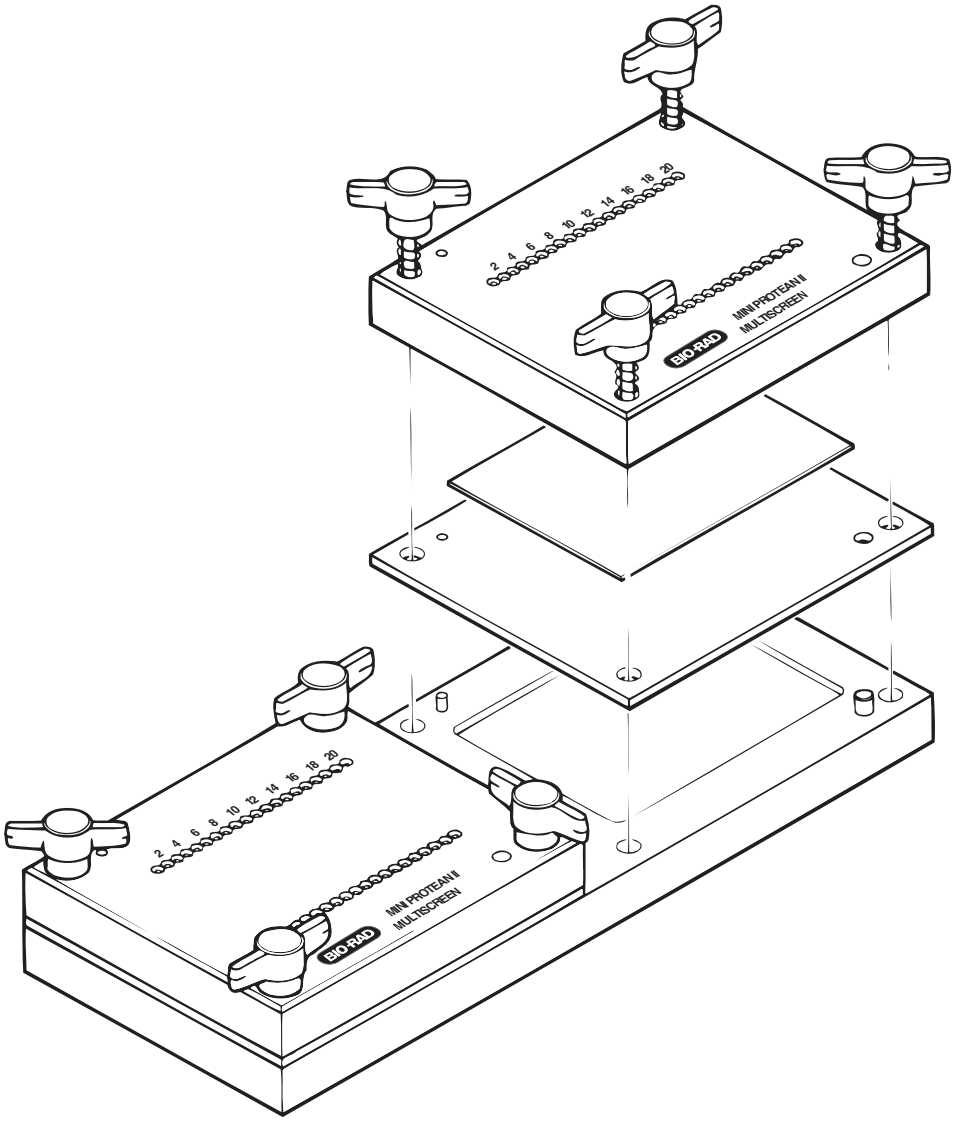


Fig. 1. Assembly of the multiscreen apparatus.

3. Lay the blocked blot on the gasket with the antigen side facing up. Center the membrane so that the channels of the sample template cover the length of the blotted sample.

4. Place the sample template on top of the membrane. The guide pins insure that the template will be properly aligned. Finger tighten the four screws. When tightening the screws, use a diagonal crossing pattern to insure even pressure on the membrane surface (see Figure 2). The multiscreen apparatus is ready for sample application.

   Note: Use of excessive force when tightening the screws is not necessary to prevent well-to-well leakage. Finger tightening is sufficient to obtain a good seal. Overtightening can cause the channels to cut into the membrane.

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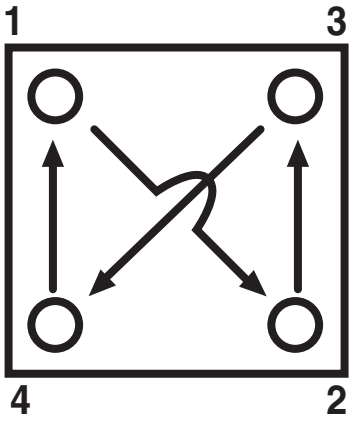


Fig. 2. Diagonal crossing pattern for tightening screws in the multiscreen apparatus.

4.3 Sample Loading and Washing

Detailed instructions for performing immunoassays, including a comprehensive troubleshooting guide, can be found in any of the Immun-Blot assay instruction manuals. See page 2 for a complete listing of Immun-Blot assay kits available from Bio-Rad.

1. To load an antibody or serum sample, tilt the multiscreen apparatus toward you so that the back of the unit is tilted up ~30° (see Figure 3). Using a syringe or Eppendorf pipet, load the solution into the bottom unmarked holes of the channels. Slow, careful delivery of sample is necessary to avoid trapping bubbles inside the channels. Titling the apparatus helps the bubbles rise to the top, towards the numbered holes of the channels. Fill the channel with 600 µl antibody solution.

   Note: Antibody buffers containing BSA or BLOTTO* are recommended for use with the multiscreen apparatus. Do not use antibody buffers with gelatin, as this may cause coagulation of gelatin within the channels of the unit.

   * BLOTTO is an acronym for Bovine Lacto Transfer Technique Optimizer, and refers to non-fat dry milk.[1,2]

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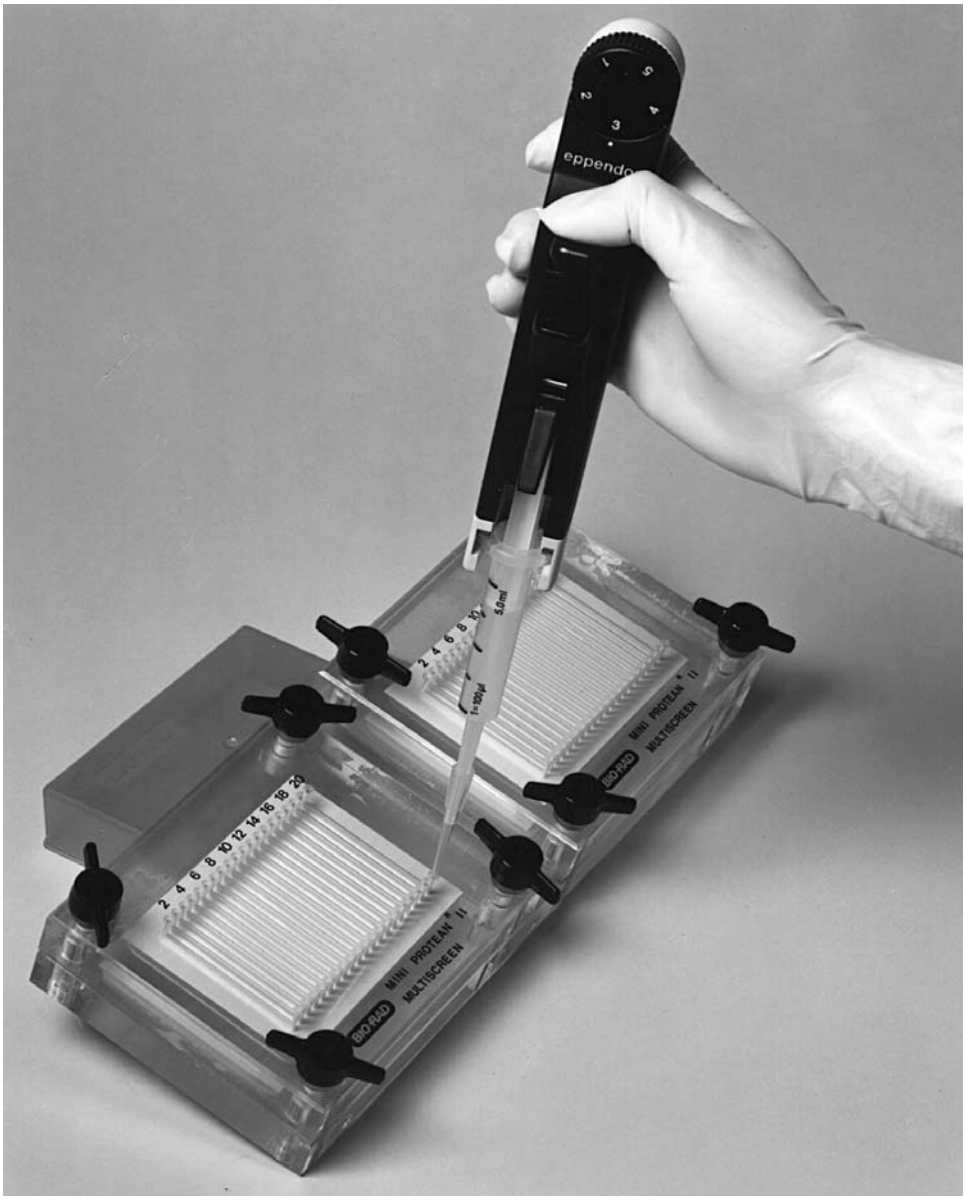


Fig. 3. Tilt the multiscreen apparatus toward you during sample application.

2. Wash solutions can be applied in the same manner as the antibody samples, or with the Eppendorf repeater pipet. Use 600 µl per channel. The number and stringency of washes may vary and should be determined separately for each experiment. However, a minimum of three washes with a buffer containing a detergent such as Tween-20 is recommended after each antibody incubation. See Section 5 for buffer formulations.

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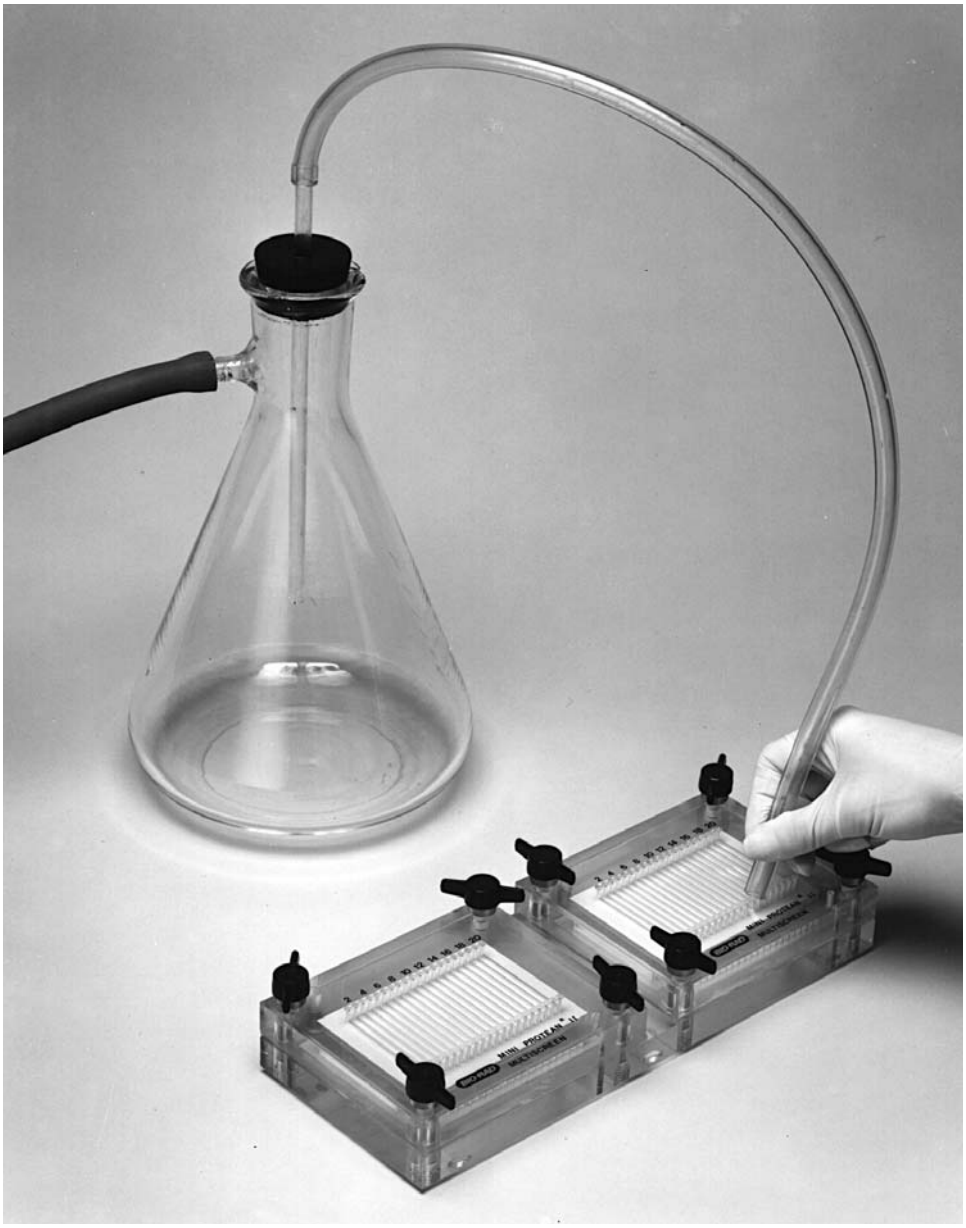


Fig. 4. Vacuum aspiration of sample and wash solutions.

3. Antibody samples and wash solutions can be rapidly removed by vacuum aspiration. The tubing from the vacuum source should be attached to the bottom, unmarked row of holes. Move the tubing back and forth along the row of holes until all the channels are dry (see Figure 4). To save an antibody or serum sample after incubation, remove the solution individually from the channel with a pipet or syringe.

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4.4 Color Development of Enzyme Conjugates

Incubation of the blot with antibodies conjugated to enzymes, such as horseradish peroxidase or alkaline phosphatase, can be conducted either in the multiscreen apparatus or in a separate vessel. If this step is carried out in the unit, wash the blot in the apparatus after the second antibody incubation as outlined in Section 4.3.

1. Color development of enzyme conjugated antibodies should be performed in a separate container to prevent permanent discoloration of the multiscreen apparatus. Remove the membrane by loosening the four screws. Lift out the sample template and move the membrane to a color development vessel.
2. Wash the membrane once with TBS for 5 minutes. After the color development solution has been prepared, incubate the membrane in the solution. Gently agitate the solution until development is complete. Remove the solution and rinse the membrane several times in distilled water to stop the reaction. Air dry the blot on filter paper.

4.5 Detection with Colloidal Gold Conjugates

Incubation of the blot with colloidal gold conjugated antibodies, protein A, or protein G, should be conducted in a separate vessel to prevent discoloration of the multiscreen apparatus.

1. Remove the blot from the multiscreen apparatus after washing to remove excess first antibody. Place the membrane in a color development vessel.
2. Wash the membrane once with TBS for 5 minutes. Add the gold solution to the vessel until the membrane is completely covered. Gently agitate the solution. Red bands identifying antigen will appear on the membrane surface within 10–15 minutes at the sites of highest antigen concentrations. Allow the incubation to continue until the desired sensitivity is achieved.

Section 5
Solutions for Immunoassay Applications

Tris Buffered Saline, 1 x TBS, 2 L
20 mM Tris-HCl, 500 mM NaCl, pH 7.5
Dissolve 4.84 g Tris, 58.48 g NaCl in ~1.5 L distilled, deionized H_2O. Adjust to pH 7.5 with HCl. Adjust the volume to 2 L with dd H_2O.

Note: Bio-Rad's Premixed Tris-Buffered Saline (catalog number 170-6430) eliminates weighing of buffer components. One bottle produces 1 L of 10 x TBS.

Tween-20 Wash Solution, 1X TTBS, 1 L
20 mM Tris-HCl, 500 mM NaCl, 0.05% Tween-20, pH 7.5
Add 0.5 ml Tween-20 to 1 L of TBS.
Blocking Solution, 100 ml

Both of the following blocking solutions can be used with nitrocellulose. The solutions containing BLOTTO should be used with Zeta-Probe membrane. Incubate nitrocellulose blots for 1 hour at room temperature. Zeta-Probe membrane should be blocked for 2 hours at room temperature.

3% Gelatin - TBS
Add 3.0 g gelatin to 100 ml TBS. Heat to 50 °C, stirring until dissolved. A microwave oven will quickly solubilize the gelatin, but do not heat above 65 °C.

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OR:
5% BLOTTO in 100 ml of TBS
Add 5% g of BLOTTO to 100 ml of TBS.
Antibody Buffer, 200 ml
1% BSA - TTBS
Add 2 g BSA to 200 ml TTBS. Stir to dissolve.

OR
1% BLOTTO in TTBS.
Add 2 g of BLOTTO to 200 ml of TTBS.

First Antibody Solution

Dilute antigen specific primary antibody to the appropriate titer in antibody buffer.

Second Antibody Solution, 100 ml

Dilute species specific Bio-Rad second antibody enzyme conjugate, 1:3,000 by adding 33 µl of conjugate to 100 ml of antibody buffer.

Consult the Immun-Blot assay kit instruction manual for dilution protocols of the colloidal gold conjugates.

Color Development Solution

The specific chemicals and buffers are dependent on the enzyme conjugate being used. See the Immun-Blot assay kit instruction manual for details on how to make the appropriate solution.

Section 6
References

1. Jerome, J. F. and Jaehning, J. A., *Mol. and Cell Bio.*, **6**, 1633 (1986).
2. Johnson, D. A., et. al., *Gene Anal. Tech.*, **1**, 3 (1984).

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