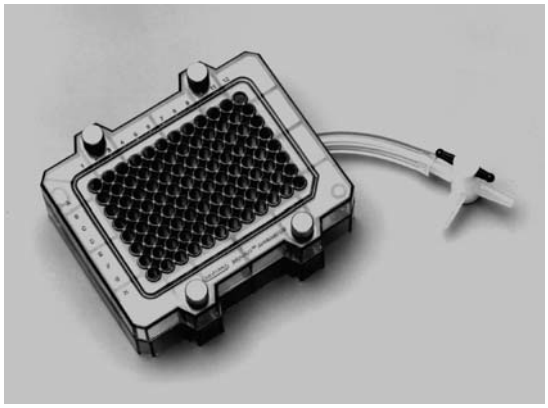

Bio-Dot® Microfiltration Apparatus

Instruction Manual

Catalog Numbers
170-6545
170-6547

BIO-RAD

For technical service, call your local Bio-Rad office or, in the U.S., call 1-800-424-6723.

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Section 1
Introduction

The Bio-Dot microfiltration apparatus can be used for any application requiring rapid immobilization and screening of unfractionated or purified proteins, nucleic acids, or macromolecular complexes on membranes, such as nitrocellulose or Zeta-Probe® membrane. The Bio-Dot apparatus is provided as a complete unit, or as a modular addition to the Bio-Dot SF slot format microfiltration apparatus. Conversion of the Bio-Dot apparatus to the Bio-Dot SF apparatus is accomplished by purchasing the Bio-Dot SF module, which provides the 48-well slot format sample template.

The Bio-Dot apparatus is simple to operate. As shown in figure 1, a sheet of membrane is clamped between the gasket and the 96-well sample template. The gasket is aligned above the support plate, which is placed over the vacuum reservoir. This assembly is attached to a vacuum source by the in-line 3-way flow valve, which allows on/off control of vacuum during assay procedures. The entire assembly is held together by the four screws on the sample template, and the patented rubber sealing gasket seals prevent well-to-well leakage, whether the vacuum is on or off. Sample can be easily applied to the 96-well formate with a standard pipet or with the Costar Octapette pipet. The material used in the construction of the Bio-Dot blotting apparatus can withstand rigorous sterilization and cleanup procedures. The Bio-Dot apparatus can be repeatedly autoclaved, and is resistant to many chemicals, including acids, bases, and ethanol.

1.1 Specifications

Materials	
Bio-Dot apparatus	Molded polysulfone
Bio-Dot gasket	Silicone rubber
Stopcock	Polytetrafluoroethylene (PTFE)®
Tubing	Tygon®
Shipping weight	600 grams
Overall size	13 x 15 x 6 cm
Membrane size	12 x 9 cm sheet
Autoclaving	15 minutes at 250°F (121°C) with a 1 minute fast exhaust
Chemical compatibility	The Bio-Dot apparatus can be used with 100% alcohol solutions and concentrated alkali or acid solutions. It cannot be used with aromatic or chlorinated hydrocarbons. (See table 1)

Section 2
Special Handling Features

The Bio-Dot apparatus withstands autoclave temperatures for sterilization, as well as cleaning with alcohols, acids, and base solutions.

2.1 Autoclaving

The Tygon tubing and flow valve cannot be autoclaved. All other components of the apparatus withstand the autoclave treatment. After repeated autoclaving (~25 cycl es), the silicone rubber gasket may need replacing. The autoclave conditions that should be used are a maximum sterilization temperature of 250°F (121°C) for 15 minutes, followed by a 1 minute fast exhaust. Higher temperatures or increased exposure times will significantly reduce the life of the apparatus. Do not autoclave the unit with the thumbscrews tightened, as this may cause the unit to warp during exposure to the elevated temperatures.

2.2 Chemical Stability

The apparatus is stable in acid and base solutions, as well as alcohol solutions. This feature allows rapid cleanup and sterilization of the apparatus and gaskets. The unit is not compatible with polar, aromatic, or chlorinated hydrocarbons, esters, and ketones. These solvents will cause degradation of the plastic. See Table 1 for list of chemical stabilities. For color development in the apparatus, the unit is compatible with both the methanol used in horseradish peroxidase (HRP) color development systems and the low concentration of dimethyl formamide (DMF) used to solubilize the alkaline phosphatase (AP) color development reagents. However, high concentrations of DMF will attack the plastic. Also, the unit is completely compatible with the low concentrations of diethyl pyrocarbonate (DEP) used as an alternative to autoclaving for elimination of RNase activity.

Table 1. Chemical Compatibility

Chemicals compatible with Bio-Dot apparatus	
Hydrochloric acid	Methanol
Sulfuric acid	Ethanol
Phosphoric acid	Butanol
Glacial acetic acid	Isopropyl alcohol
Sodium hydroxide	Formaldehyde
Potassium hydroxide	Hydrogen peroxide
Ammonium hydroxide	Ethylene glycol
Heptane	5% acetone in H_2O
Nitric acid	
Chemicals incompatible with Bio-Dot apparatus (use voids warranty)	
Ethyl acetate	Toluene
Butyl acetate	Benzene
Acetone	Methyl ethyl ketone
Chloroform	Methylene chloride
Trichloroacetic acid	

Section 3
Bio-Dot Assembly

3.1 Assembly

1. Clean and dry the Bio-Dot apparatus and gasket prior to assembly.
2. Place the gasket support plate into position in the vacuum manifold. (There is only one way to slide the plate into the manifold.)
3. Place the sealing gasket on top of the gasket support plate. The guide pins on the vacuum manifold help align the 96 holes in the gasket over the 96 holes in the support plate. Visually inspect the gasket to make sure the holes are properly aligned. If the gasket is not centered, pull lightly at the corners until it is aligned.

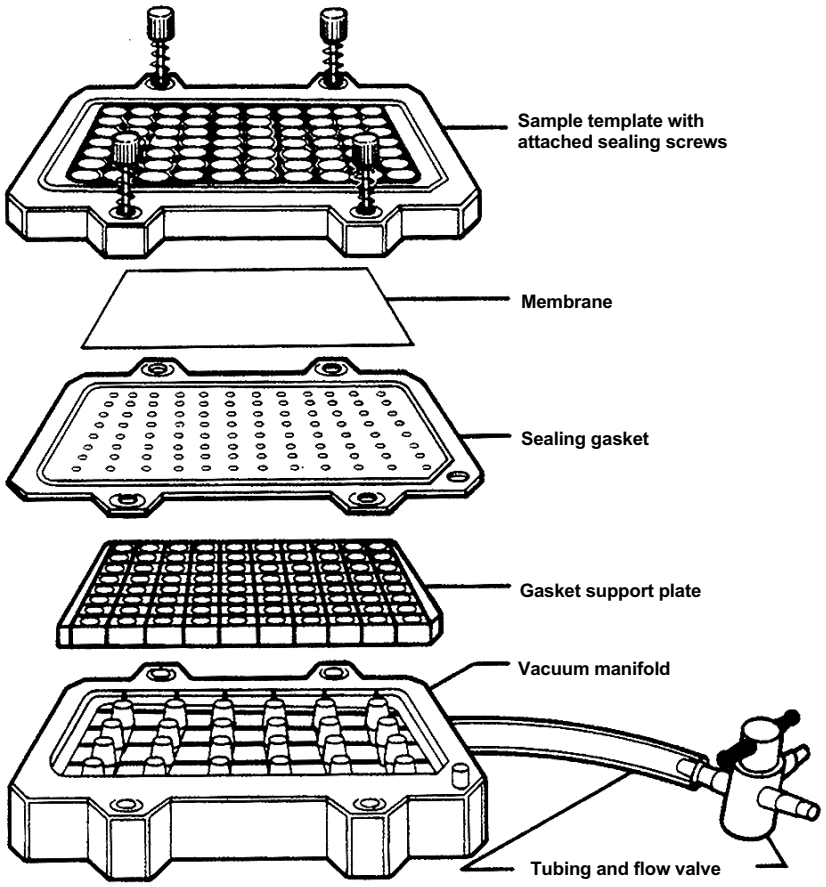


Fig. 1. Diagram of proper Bio-Dot apparatus assembly.

4. Always use forceps or wear gloves when handling membranes. Prewet the nitrocellulose or Zeta-Probe® membrane by slowly sliding it at a 45° angle into wetting solution. Note: PVDF membrane is not recommended. Wet nitrocellulose in 6x sodium, sodium citrate (SSC) for nucleic acid applications, and in tris-buffered saline (TBS) for protein blotting. Wet Zeta-Probe membrane in distilled water. See Sections 9 and 10 for solution preparation. A 10 minute soak is recommended for complete wetting of the membrane to insure proper drainage of solutions. Remove the membrane from the wetting solution. Let the excess liquid drain from the membrane. (Touching the membrane to a sheet of filter paper is a simple method for removing excess buffer.) Lay the membrane on the gasket in the apparatus so that it covers all of the holes. The membrane should not extend beyond the edge of the gasket after the Bio-Dot apparatus is assembled. Remove any air bubbles trapped between the membrane and the gasket.

   Note: PVDF membrane is not recommended.

5. Place the sample template on top of the membrane. The guide pins ensure that the template will be properly aligned. Finger–tighten the four screws. When tightening the screws, use a diagonal crossing pattern to ensure uniform application of pressure on the membrane surface (see Figure 2).

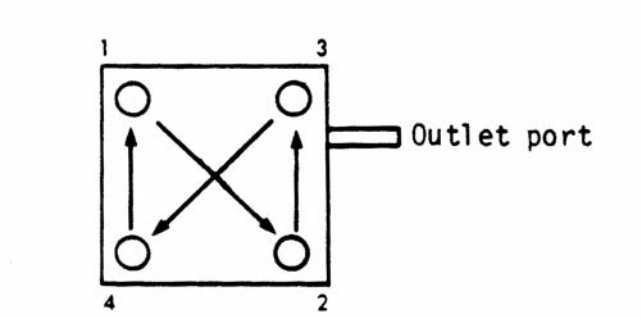
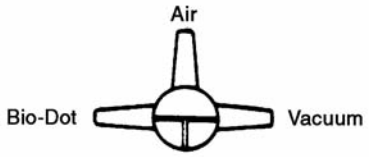


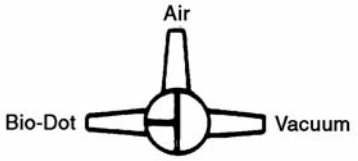
Fig. 2. Diagonal crossing pattern for tightening screws in the Bio-Dot apparatus.

6. Attach a vacuum source (house vacuum or a vacuum pump) to the flow valve with a waste trap set up and positioned between the vacuum outlet and the flow valve. Turn on the vacuum and set the 3-way valve to apply vacuum to the apparatus (flow valve setting 1, Figure 3).
7. With vacuum applied, repeat the tightening process using the diagonal crossing pattern. Tightening while vacuum is applied ensures a tight seal, preventing cross contamination between slots. **Failure to tighten screws during application of vacuum prior to starting the assay may lead to leaking between the wells.**
8. Adjust the flow valve so that the vacuum manifold is open to air pressure (flow valve setting 2, Figure 3). Apply 100 µl buffer to all 96 sample wells. Use of the 8-channel pipet and buffer reservoirs (see Section 13 for ordering information) will simplify the process of adding solutions to the Bio-Dot apparatus. Addition of buffer is necessary to rehydrate the membrane following the vacuum procedure in step 7. If this step is not performed prior to applying samples, assay results will show halos or weak detection signal.
9. Gently remove the buffer from the wells by vacuum (flow valve setting 3, Figure 3). **Watch the sample wells**. As soon as the buffer solution drains from all the wells, adjust the flow valve so that the unit is exposed to air and disconnect the vacuum. At this point, the unit is ready for sample application.

Flow valve setting 1.
The vacuum manifold is exposed to the vacuum source only. Use for applying vacuum to the Bio-Dot apparatus.



Flow valve Setting 2.
The manifold is exposed to air.
Use for gravity filtration procedures.



Flow valve Setting 3.
The manifold is exposed to both air and the vacuum. Use this setting for gentle vacuum applications where the amount of vacuum is regulated by putting a finger over the port exposed to the atmosphere.

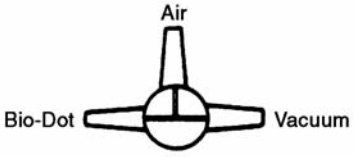


Fig. 3. Optional settings for the 3-way flow valve to obtain optimal performance from the Bio-Dot apparatus.

3.2 Helpful Hints

1. During the assay, do not leave the vacuum on. This may dehydrate the membrane and may cause halos around the wells. Apply vacuum only until solutions are removed from the sample wells, then adjust the flow valve so that the unit is exposed to air and disconnect the vacuum.
2. If some sample wells are not used in a particular assay, those wells must be closed off to ensure proper vacuum to the wells in use. There are three ways to close off unused wells. One is to apply a 3% gelatin solution to those wells. Gelatin will clog the membrane and cut off the vacuum flow to the clogged wells. The second method is to cover the unused portion of the apparatus with tape to prevent air from moving through those wells. The third method is to add buffer to the empty wells at each step instead of sample or wash solutions.
3. If an overnight incubation is desired, adjust the flow valve so that the vacuum manifold is closed off from both the vacuum and air before applying samples (see Figure 3). In this configuration, solutions will remain in the sample wells with less than 10% loss of volume during an overnight incubation. Note that the unit must be kept at a constant temperature during extended incubations. If the unit cools more than 10°C (20°F), a partial vacuum will build inside the unit and drainage will occur.
4. Any particulate in samples or solutions will block the membrane and restrict flow of solutions through the membrane. For best results, filter or centrifuge samples to remove particulate matter.

5. Check the wells after sample has been applied to ensure that there are no air bubbles in the wells. Air bubbles will prevent the sample from binding to the membrane. Air bubbles may be removed by pipetting the liquid in the well up and down.
6. Proper positioning of the flow valve relative to the level of the apparatus is important for proper drainage. The speed of filtration is determined by the difference in hydrostatic pressure between the fluid in the sample wells and the opening of the flow valve which is exposed to air. If the opening of the flow valve is above the level of the sample wells, very little drainage will occur. When the flow valve is positioned at a level below the sample wells, proper drainage will occur during filtration applications.
7. The best method for removing the blotted membrane from the BioDot apparatus is to leave the vacuum on following the wash step. With the vacuum on, loosen the screws and remove the sample template. Next, turn off the vacuum and remove the membrane.
8. A method for applying gentle vacuum to the apparatus is to adjust the flow valve so that it is open to air, the vacuum source, and the vacuum manifold, while the vacuum is on. Then, use a finger to cover the valve port exposed to the atmosphere. The amount of vacuum reaching the manifold will be regulated by the pressure of your finger on the valve.
9. For applications using glass membranes that might break under vacuum pressure, an extra piece of tubing can be attached to the flow valve to increase hydrostatic pressure during wash steps. This tubing should extend approximately 2–3 feet below the level of the apparatus, usually to a waste receptacle on the floor. With this increased hydrostatic pressure, fluid will drain from the apparatus in 3–4 minutes. This type of gentle pressure is also useful for binding nucleic acids to nitrocellulose or Zeta-Probe membranes.

Section 4
Protein Blotting

4.1 Immunoassay Procedure

Detailed instructions, including a comprehensive troubleshooting guide, for performing immunoassays are included in the Immun-Blot® instruction manuals.

1. Assemble the Bio-Dot apparatus as described in Section 3. Prewet the membrane prior to placing it in the apparatus. Nitrocellulose membranes are prewetted in TBS; nylon membranes, such as the Zeta-Probe membrane, are prewetted in distilled water (see Section 9 for solution preparation). Make sure that all the screws have been tightened under vacuum to ensure that there will not be any cross-well contamination.

   Notes: Zeta-Probe membranes must be removed from the Bio-Dot apparatus after the antigen is immobilized. The blocking and other incubation steps should be carried out in a separate container. Zeta-Probe membranes require more stringent blocking conditions, using 5% (w/v) BLOTTO or 3% (w/v) gelatin in 1x TBS, which cannot be filtered through the membrane using the Bio-Dot apparatus.

2. Rehydrate the membrane to ensure uniform binding of the antigen. Use 100 µl TBS per well for nitrocellulose membranes. Use 100 µl distilled water per well for Zeta-Probe membranes.

3. Adjust the flow valve so that the vacuum chamber is open to air (flow valve setting 2, Figure 3). Fill the appropriate wells with antigen (protein) solution using any volume up to 500 µl per well. Multiple applications of antigen to a sample well are possible, but the most rapid and efficient use of the apparatus is achieved by applying the required amount of antigen in a minimal sample volume.

4. Allow the entire sample to filter through the membrane by gravity flow. Make sure that the flow valve is positioned at a level below the sample wells to ensure proper drainage during filtration applications. This passive filtration is necessary for quantitative antigen binding. Each well should be filled with the same volume of sample solution to ensure homogenous filtration of all sample wells. Generally, it takes 30–40 minutes for 100 µl of the antigen solution to filter through the membrane. If antigen is very dilute, and it is necessary to ensure that all proteins in the applied sample are filtered through the membrane, an optional wash step can be performed. To perform this wash, add an aliquot of TBS equal to the original sample volume to each sample well. Allow this material to passively filter through the membrane by gravity filtration. (If the membrane is going to be removed from the apparatus following binding of antigen, proceed to step 6 and follow the instructions for the wash step. The wash step should be performed prior to disassembling the apparatus to ensure that all antigen is removed from the drain ports underneath the membrane.)

5. After the antigen samples have completely drained from the apparatus, add 200–300 µl of the blocking solution to each well. Allow gravity filtration to occur until the blocking solution has completely drained from every well. This step should take approximately 60 minutes. Do not apply vacuum to speed up this step, as it will lead to poor assay results.

6. Adjust the flow valve so that the vacuum chamber is exposed to air. Add 200–400 µl of the Tween, tris-buffered saline (TTBS) wash solution to each well. Adjust the flow valve to the vacuum position and pull the wash solution through the membrane. Disconnect the vacuum as soon as the wash solution has drained from all the sample wells. Repeat the wash step. If the membrane is to be removed from the apparatus prior to performing an immunoassay, remove it at this point. Otherwise, proceed to step 7. Note: For better results with Zeta-Probe, use 0.3% Tween 20.
7. Open the flow valve to air. Add 100 µl of primary antibody solution to each sample well. Allow gravity filtration to occur until the antibody solution has completely drained from the sample wells (approximately 30–40 minutes).
8. Apply vacuum to the apparatus to remove any excess liquid from the sample wells.
9. Open the flow valve to the atmosphere and add 200–400 µl of TTBS wash solution to each well. Apply vacuum until the wash solution is drained from the wells. Repeat for a total of three wash cycles.
10. With the vacuum off and the flow valve open to air, add 100 µl of secondary antibody solution to each well. Allow gravity filtration to occur (30–40 minutes) until all solution has drained from the wells.
11. Turn the vacuum on and drain the wells. Add 200–400 µl of TTBS wash solution to each well and drain completely. Repeat for a total of two washes.

    Note: At this point, the membrane is ready for development. Color development of enzyme conjugated antibodies can be performed in the apparatus or in a separate reservoir. If performing autoradiography, remove the membrane, dry it on a filter paper, wrap it with plastic wrap, and expose it to X-ray film. The best method to remove the membrane from the Bio-Dot apparatus is to leave the vacuum on following the last wash step. While the vacuum is on, loosen the screws and remove the sample template. Turn off the vacuum and remove the membrane.
12. For color development in a separate vessel, remove the membrane and place it in the color development vessel. Wash the membrane twice with TBS to remove excess Tween 20. Prepare the color development solution, and incubate the membrane in the solution. Gently agitate the solution until development is complete. When the reaction has developed, remove the membrane and rinse it in distilled water to stop the reaction. Place the membrane on filter paper to airdry.
13. When using HRP color development substrate, wash each well twice with 200 µl TBS to eliminate excess Tween 20. This wash step is not necessary when using AP systems or NBT/BCIP color development. Add 100–200 µl of the color development solution to each well. The reagent can be allowed to react while the solution slowly drains by gravity filtration or the reaction time can be extended by closing the flow valve prior to adding the substrate. In either application, when the color development is completed, the excess substrate should be removed by vacuum and all the sample wells should be vacuum washed with 200 µl of distilled water to stop the reaction. Following this wash step, remove the membrane from the apparatus. Rinse the membrane in distilled water and allow it to airdry on filter paper.

4.2 Special Protein Blot Applications

1. Soluble enzyme substrate reactions and quantitations

   Perform an immunassay as described in Section 4.1. Prior to color development, disconnect the vacuum and close the flow valve. Add an equal volume of substrate solution to all wells. Visualize positive reactions and record. For quantitation, withdraw equal aliquots of the soluble substrate reactant from each well and transfer to a plastic disposable microplate. Quantitate using Bio-Rad's Model 620 video densitometer.

2. Assay for particular antigen or target cell antigen

   a. Place a prewetted filter paper (Whatman GF/B) in the Bio-Dot apparatus. Attach the sample template and tighten the screws. Fill all the wells with buffer and apply a vacuum. With the vacuum on and the buffer draining, retighten the screws. The presence of buffer while applying vacuum will help prevent the filter paper from breaking. When the buffer is gone, turn off the vacuum and close the flow valve.

   b. Add 50 µl fetal bovine serum (FBS), 10% v/v in blocking buffer. Allow the FBS buffer to incubate for 10 minutes, then open the flow valve and filter through by gravity.

   c. Add approximately 12,500 target cells in 50 µl FBS buffer to each well. Gently pull the solution through the membrane by attaching tubing to the flow valve to increase the hydrostatic pressure (see Section 3.2). Perform three washes with TBS buffer using tubing rather than vacuum to speed the flow rate.

   d. Perform antibody incubations as described in Section 4.1 for protein immunoassays.

Section 5
DNA Blotting

This section gives protocols for DNA blotting. The alkaline blotting method, using Zeta-Probe® membrane, and the standard method for DNA blotting to nitrocellulose is described.

1. The target DNA must be denatured prior to application to the membrane. When using the Zeta-Probe membrane, denature the DNA sample by addition of NaOH and EDTA solution to final concentrations of 0.4 M NaOH, 10 mM EDTA. Heat the sample to 100°C for 10 minutes to ensure complete denaturation. When applying DNA to A nitrocellulose membrane, denature the DNA in the same manner. The DNA must then be neutralized by adding an equal volume of cold 2 M ammonium acetate, pH 7.0 to the target DNA solution.
2. Pre-wet the membrane by placing the membrane gently at a 45° angle into a tray of the wetting solution. Always wear gloves when handling blotting membranes. Nitrocellulose membranes should be wetted in 6x SSC; Zeta-Probe membranes should be wetted in distilled water (see Section 10 for recipes).
3. Assemble the Bio-Dot apparatus according to the instructions in Section 3.1. Apply the vacuum and then retighten the screws that hold the apparatus together. Rehydrate the membrane with 500 µl Tris-EDTA (TE) or H_2O, as described in Section 3.1. At this point, the unit is ready for sample application.
4. Samples and wash solutions should be applied with a standard pipet or a Costar Octapette pipet with the vacuum off and the flow valve open. Apply the denatured DNA in a 50–500 µl sample volume. Multiple loadings may be performed. However, best binding and most rapid results occur using minimum sample volumes. Fill all wells with the same volume to obtain homogeneous filtration.
5. The sample may be pulled through by applying a gentle vacuum, or by gravity filtration.

   Notes: a method for applying gentle vacuum to the apparatus is to adjust the flow valve to setting 3. Use a finger to cover the valve port exposed to air. The amount of vacuum reaching the manifold will be regulated by the pressure of your finger on the valve.
6. After the sample has filtered through, add 500 µl 0.4 M NaOH to each well for Zeta-Probe membrane, or 2x SSC for nitrocellulose. Apply the vacuum by setting the 3-way valve to setting 1 until the sample wells are empty.
7. Disassemble the Bio-Dot apparatus. Remove the blotted membrane and rinse it in 2x SSC. Allow the membrane to airdry. The Zeta-Probe membrane is ready for hybridization immediately after air-drying. If hybridization is not to be undertaken within 2 days, then vacuum-bake the blotted Zeta-Probe membrane at 80°C for 30 minutes. Nitrocellulose membrane must be baked under vacuum for 2 hours at 80°C before hybridization. The Zeta-Probe membrane and nitrocellulose membranes can be stored dry between two pieces of filter paper in plastic bags at 23–25°C.

Section 6
RNA Blotting

RNA must be denatured prior to application to Zeta-Probe® or nitrocellulose membranes to ensure optimal hybridization. Two protocols are presented for denaturing RNA samples.

6.1 Alkaline RNA Denaturation and Fixation

1. Always wear gloves when handling blotting membranes. Pre-wet the blotting membrane by placing it gently at a 45° angle into a tray of wetting solution. The Zeta-Probe membrane is wetted in distilled water, nitrocellulose is wetted in 6x SSC (see Section 9 for solution preparation).
2. Assemble the Bio-Dot apparatus according to the instructions in Section 3.1. Remember to apply the vacuum and then retighten the screws that hold the apparatus together.
3. Immediately before use, dissolve RNA samples in 500 µl of ice-cold 10 mM NaOH, 1 mM EDTA.
4. Samples and wash solutions may be applied with a standard pipet or a Costar Octapette pipet. Apply the denatured RNA, and pull the sample through by passive filtration or by applying a gentle vacuum.

   Note: A method for applying gentle vacuum to the apparatus is to adjust the flow valve to setting 3. Use a finger to cover the valve port exposed to air. The amount of vacuum reaching the manifold will be regulated by the pressure of your finger on the valve.
5. Rinse all wells to wash through any sample on the side of the wells. Rinse with 500 µl cold 10 mM NaOH, 1 mM EDTA. Apply vacuum (flow valve setting 1, Figure 3) until the sample wells are dry.
6. Disassemble the Bio-Dot apparatus. Remove the blotted membrane and rinse it in 2x SSC, 0.1% sodium dodecyl sulfate (SDS). Nitrocellulose membranes must be baked under vacuum for 2 hours at 80° before hybridization. The Zeta-Probe membrane is ready for hybridization. If hybridization is not to be undertaken within 2 days, then bake the Zeta-Probe membrane under vacuum for 30 minutes at 80°C. The Zeta-Probe membrane and nitrocellulose membranes can be stored dry between two pieces of filter paper in plastic bags at 23–25°C.

6.2 Glyoxal RNA Denaturation and Fixation

1. Prepare RNA samples to the following final concentrations:

   50% dimethyl sulfoxide (DMSO)

   10 mM NaH_2PO_4, pH 7.0

   1 M glyoxal
2. Incubate the RNA for 1 hour at 50°C. Cool the samples on ice.
3. Always wear gloves when handling blotting membranes. Prewet the blotting membrane by placing it gently at a 45° angle into a tray of wetting solution. The Zeta-Probe membrane is wetted in distilled water, nitrocellulose is wetted in 6x SSC (see Section 10 for solution preparation).

4. Assemble the Bio-Dot apparatus according to the instructions in Section 3.1. Remember to apply the vacuum and then retighten the screws that hold the apparatus together.
5. Samples and wash solutions may be applied with a standard pipet or a Costar Octapette pipet. Apply the denatured RNA, and pull the sample through by passive filtration or by applying a gentle vacuum.

   Note: a method for applying gentle vacuum to the apparatus is to adjust the flow rate valve to setting 3. Use a finger to cover the valve port exposed to air. The amount of vacuum reaching the manifold will be regulated by the pressure of your finger on the valves.
6. Rinse all wells to wash through any sample on the side of the wells. Rinse with 500 µl TE. Apply vacuum (flow valve setting 1, Figure 3) until the sample wells are dry.
7. Disassemble the Bio-Dot apparatus. Remove the blotted membrane.
8. Remove glyoxal adducts by pouring 20 mM Tris-HCl, pH 8.0, 1 mM EDTA heated to 95°C onto the membrane and agitating at room temperature until the solution cools. Place the membrane in a vacuum oven at 80°C for 1 hour for the Zeta-Probe membrane, 2 hours for nitrocellulose.
9. Nitrocellulose membrane must be baked under vacuum for 2 hours at 80°C before hybridization. If hybridization is not to be undertaken within 2 days, then bake the Zeta-Probe membrane under vacuum for 30 minutes at 80°C. The Zeta-probe membrane and nitrocellulose membranes can be stored dry between two pieces of filter paper in plastic bags at 23–25°C.

Section 7
Hybridization Protocols for Nucleic Acids

7.1 Probe Recommendations

The specific activity, concentration, size range, and purity of the probe all have an important effect on signal-to-noise ratio during hybridization. For hybridization on Zeta-Probe membrane, the following is recommended:

Probe specific activity:	10^8 cpm/µg probe
Probe concentration in the hybridization mixture:	10^6 counts/ml (10–50 mg/ml)
Probe length:	200–1,000 bp

Optimal probe specific activity and concentration can vary according to available hybridization sites and exposure time. Alternative hybridization protocols are necessary when probe lengths vary outside this recommended range. (Bio-Rad Laboratories 1987)

Probe cleanup is essential to minimize background. Unincorporated nucleotides present after probe preparation contribute to hybridization background. The most effective cleanup method is by column chromatography. This can be done quickly and easily with the Bio-Spin® chromatography columns (Bio-Spin 6 columns, catalog number 732-6000, or Bio-Spin 30 columns, catalog number 732-6004).

After cleanup, denature double-stranded probes by heating to 95–100°C for 5 minutes. Then cool rapidly on ice. Use the probe as soon as possible after preparation.

There are several hybridization protocols given in this section. All protocols are for using DNA probes to hybridize to either DNA or RNA. The 7% SDS hybridization protocol requires minimal prehybridization treatment and has a high signal strength and low background. Further references and techniques for hybridizing to the Zeta-Probe membrane may be found in the Zeta-Probe membrane instruction manual.

The final volume of hybridization solution is important in reducing background. For prehybridization and hybridization, use 150 µl solution/cm^2 of membrane. For washes, use at least 350 µl/cm^2 of membrane.

7.2 Hybridization Protocols for DNA or RNA bound to Nitrocellulose or Zeta-Probe Membrane

Prehybridization

1. Place the blotted membrane inside a heat-sealable plastic bag. Seal three sides, leaving the top side open.
2. Pipet in the correct prehybridization solution for application:

For DNA or RNA Bound to Zeta-Probe Membrane (Bio-Rad Laboratories 1987)	**For DNA Bound to Nitrocellulose (Maniatis et al. 1982)**	**For RNA Bound to Nitrocellulose (Thomas 1980)**
1 mM EDTA	6x SSC	50% formamide
7% SDS	0.5% SDS	5x SSC
0.5 M $NaHPO_4$, pH 7.2	5x Denhardt's solution	1x Denhardt's solution
	100 µg/ml denatured salmon sperm DNA	50 mM $NaHPO_4$, pH 6.5
	1 mM EDTA	250 µg/ml denatured salmon sperm DNA

The carrier DNA used with nitrocellulose must be denatured before adding it to the prehybridization solution. Heat the DNA at 100°C for 5 minutes and cool rapidly.

3. Seal the top of the bag and incubate.

For DNA or RNA Bound to Zeta-Probe Membrane	For DNA Bound to Nitrocellulose	For RNA Bound to Nitrocellulose
5 minutes at 65°C	2–4 hours at 68°C	8–20 hours at 42°C

Hybridization

1. Cut one corner of the plastic bag. Remove the prehybridization solution and replace it with a fresh batch of the same solution, except when binding RNA to nitrocellulose. In that case, add 10% dextran sulfate to the hybridization solution.

   Note: formamide can also be used in the hybridization buffer to lower the incubation temperature when binding DNA to nitrocellulose or Zeta-Probe membrane. (Maniatis et al. 1982, Casey and Davidson 1977)For alternative protocols, see the Zeta-Probe Membrane Instruction Manual.

2. Add the denatured probe, remove all air bubbles and reseal the bag. Mix the contents of the bag. Hybridize with agitation.

For DNA or RNA Bound to Zeta-Probe Membrane	For DNA Bound to Nitrocellulose	For RNA Bound to Nitrocellulose
4–24 hours at 65°C	4–24 hours at 68°C	4–24 hours at 42°C

3. Carefully remove the hybridization solution by cutting one corner. Remove hybridized Zeta-Probe membrane from the plastic bag.

   Note: Once hybridizationhas begun, do not let the membrane dry.

Washes

1. Agitate the solutions when washing membranes.

For DNA or RNA Bound to Zeta-Probe Membrane	For DNA Bound to Nitrocellulose	For RNA Bound to Nitrocellulose
A. Wash two times for 30–60 minutes at 65°C in: 1 mM EDTA, 40 mM $NaHPO_4$, pH 7.2 5% SDS	**A.** Rinse in: 2x SSC, 0.5% SDS	**A.** Wash 4 times at room temperature for 5 minutes in 2x SSC, 0.1% SDS
B. Wash two times for 30–60 minutes at 65°C in: 1 mM EDTA, 0.1x SSC, 0.1% SDS, 40 mM $NaHPO_4$, pH 7.2, 1% SDS	**B.** Wash at room temperature for 5 minutes in:	**B.** Wash two times at 50°C in: 2x SSC, O.5% SDS 1 mM EDTA

2. After washing, the blotted membrane is ready for autoradiography. If no further cycles of hybridization are to be done on the membrane, the membrane can be dried. When reprobing, do not allow the membrane to dry between hybridizations. Make the autoradiographic exposure with the moist membrane wrapped in plastic wrap. Do not allow the wet membrane to come in contact with the film, because a wet Zeta-Probe membrane will stick to the film, and any moisture on the film will cause artifacts (black spots).

7.3 Hybridization Protocols for RNA Probes

The following protocols are for RNA probes to DNA blots. Casey and Davidson (1977) contains protocols for RNA-RNA hybridizations.

Prehybridization

1. Place the blotted membrane inside a heat sealable plastic bag. Seal three sides, leaving the top side open.
2. Pipet in the prehybridization solution:

For DNA Bound to Zeta-Probe Membrane (Bio-Rad Laboratories 1987)	**For DNA Bound to Nitrocellulose (Jerome and Jaehning 1986)**
50% formamide	50% formamide
1.5x sodium, sodium phosphate, EDTA (SSPE)	0.1% SDS
1% SDS	5x SSPE
0.5% non fat dry milk	5x Denhardt's solution
200 µg/ml carrier RNA DNA	200 µg/ml denatured salmon sperm
500 µg/ml denatured salmon sperm DNA	

The DNA must be denatured before adding it to the prehybridization solution by heating at 100°C for 5 minutes, followed by rapid cooling in ice.

3. Seal the bag and incubate.

DNA Bound to Zeta-Probe Membrane	**DNA Bound to Nitrocellulose**
30 minutes at 50°C	4 hours at 42°C

Hybridization

1. Immediately before use, fragment and denature the probe and carrier DNA as follows. Add to the precipitated RNA probe 0.1 ml of yeast RNA (20 mg/ml), 0.5 ml of carrier DNA (10 mg/ml), and 0.6 ml of deionized formamide, mix thoroughly, and heat at 70°C for 5 minutes.
2. Cut one corner of the bag, remove the prehybridization solution, and replace it with hybridization solution.

DNA Bound to Zeta-Probe Membrane	**DNA Bound to Nitrocellulose**
50% formamide	50% formamide
1.5x SSPE	1x Denhardt's solution
1% SDS	0.1% SDS
0.5% nonfat dry milk	100 µg/ml denatured salmon sperm DNA

3. Add probe, then seal the open corner (taking care to exclude all air bubbles). Mix the contents of the bag thoroughly. Incubate at 50°C for 4–24 hours.

   Note: After beginning hybridization the membranes should not be permitted to dry.

Washes

1. At the completion of hybridization, remove the membranes from their hybridization bags into 2x SSC. Rinse briefly, then wash them sequentially with agitation for 15 minutes at room temperature in the following solutions:

   a. 2x SSC/0.1% SDS
   b. 0.5x SSC/0.1% SDS
   c. 0.1x SSC/0.1% SDS

2. For DNA bound to nitrocellulose membranes, it may be necessary to include an RNase treatment in the wash. Membranes are treated with 20 µg/ml RNase for 30 minutes at 37°C in 2x SSC. (Santzen et al. 1986)

3. After washing, the blotted membranes are ready for autoradiography. If no further cycles of hybridization are to be done on the membrane, then the membrane can be dried. When reprobing, do not allow the membrane to dry between hybridizations. Expose moist membranes between plastic wrap or enclosed in a sealable plastic bag. Do not allow a wet membrane to come in contact with the film, because a wet Zeta-Probe membrane will stick to the film, and moisture on the film can cause artifacts.

   Note: To increase the rate of hybridization, include 10% dextran sulfate (final concentration) in the hybridization solution.(Maniatis 1982) Prewarm hybridization solution to 50°C. Denature the probe and carrier as above. Special care must be taken to ensure uniform mixing of the denatured probe with the hybridization solution, since the solution is quite viscous at 50°C.

7.4 Probe Stripping and Rehybridization

If reprobing is desired, do **not** allow the membrane to dry between hybridizations. The membrane should be stripped as soon as possible after autoradiography.

1. Wash two times, 20 minutes each, in a large volume of 0.1x SSC/0.5% SDS at 95°C.
2. Check membrane for removal of autoradiography patterns by overnight exposure.

Section 8
Solutions for Protein Applications

8.1 Solutions for Nitrocellulose Membrane

Tris Buffered Saline, 1x TBS, 2 L

20 mM Tris-HCl, pH 7.5

500 mM NaCl

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 ddH_2O.

Tween 20 Wash Solution, 1x TTBS, 1 L

20 mM Tris, pH 7.5

500 mM NaCl

0.05% Tween 20

Add 0.5 ml Tween 20 to 1 L of TBS.

Blocking Solution, 100 ml

1% BSA-TBS

Add 1.0 g bovine serum albumin (BSA) to 100 ml TBS. Stir to dissolve.

Antibody Buffer, 200 ml

1% BSA-TTBS

Add 2 g BSA to 200 ml TTBS. Stir to dissolve. 100 ml should be reserved for primary antibody and an equal volume for dilution of the secondary antibody conjugate.

Primary Antibody Solution, 100 ml

Dilute antigen specific primary antibody to the appropriate titer in 100 ml of antibody buffer.

Secondary Antibody Solution, 100 ml

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

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.

8.2 Solutions for Zeta-Probe Membrane for Protein Applications

When immobilizing antigen onto the Zeta-Probe® membrane, the immunoassay must be performed in a separate container following removal of the membrane from the Bio-Dot apparatus. Two methods of blocking are given: **Method A uses nonfat dry milk** (Jerome and Jiehning 1986, Johnson et al. 1984) as the blocking agent. **Method B uses gelatin and 1-methyl-2-pyrrolidinone (MPO)** as the blocking agents. The solutions for the two methods are not interchangeable. If Method A is chosen, all solutions must be prepared according to Method A; if Method B is chosen, all solutions must be prepared according to Method B.

TBS, Tris Buffered saline, 2 L

Same as nitrocellulose membrane solution

TTBS, Tween 20, Tris buffered saline, 2 L

Method A. Add 3 ml Tween 20 to 1 L of TBS. This solution is used when nonfat dry milk is the blocking agent.

OR:

Method B. Add 2 ml Tween 20 and 50 ml MPO to 1 L of TBS. This solution is used when gelatin and MPO are the blocking agents.

Blocking solution, 100 ml

Method A. Add 5 g of nonfat dry milk to 100 ml of TBS.

OR:

Method B. Add 3 g of gelatin to 100 ml of TBS. Warm to 37°C to dissolve the gelatin with stirring. Cool before use.

Antibody buffer, 200 ml

Method A. Add 10 g of nonfat dry milk to 200 ml TTBS. 100 ml is used for the primary antibody solution and 100 ml is used for conjugate dilution.

OR:

Method B. Add 2 g gelatin to 200 ml TTBS that already contains 5% MPO. Warm to 37°C to dissolve gelatin and cool before adding antibody. 100 ml is used for the primary antibody solution and 100 ml is used for conjugate dilution.

Primary antibody solution, 100 ml

Same as nitrocellulose membrane antibody buffer solution.

Secondary antibody solution, 100 ml

Same as nitrocellulose membrane antibody buffer solution.

Color development solution

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

Section 9
Solutions for Nucleic Acid Applications

20x SSC

3 M NaCl

0.3 M trisodium citrate (FW = 294.1)

Dissolve 175.0 g NaCl and 88.2 g trisodium citrate in ddH_2O. Adjust volume to 1 L with ddH_2O.

20x SSPE

3.6 M NaCl

0.2 M $Na_2HPO_4 \cdot 7H_2O$

0.02 M EDTA

Dissolve 210.0 g NaCl, 53.6 g $Na_2HPO_4 \cdot 7H_2O$, 7.44 g EDTA ddH_2O. Adjust volume to 1 L with ddH_2O.

TE

10 mM Tris-HCl, pH 8.0

1 mM EDTA, pH 8.0

Dilute 10 ml 1 M Tris-HCl, pH 8.0 and 4 ml 0.250 M EDTA, pH 8.0 to 1 L with ddH_2O.

100x Denhardt's Solution

2% bovine serum albumin

2% polyvinylpyrrolidone

2% Ficoll

Dissolve 2.0 g BSA, 2.0 g polyvinylpyrrolidone, 2.0 g Ficoll in ddH_2O. Adjust volume to 100 ml with ddH_2O.

20% SDS

Dissolve 200.0 g SDS in ddH_2O. Adjust volume to 100 ml with ddH_2O. It may be necessary to heat to 65°C to get into solution.

1 M $NaHPO_4$ pH 7.2

Dissolve 134.0 g Na2 $HPO_4 \cdot 7H_2O$ (F.W. = 268.07) in ddH_2O. Add 4 ml 85% H_3PO_4. Adjust volume to 1 L with ddH_2O.

50% Dextran Sulfate

50% dextran sulfate

0.2% sodium azide

Dissolve 50.0 g dextran sulfate and 0.2 g sodium azide in ddH_2O. Adjust volume to 100 ml with ddH_2O. Store at 4°C.

50% Formamide

Dilute 50.0 g formamide to 100 ml with ddH_2O. Store at 4°C. Immediately before use, deionize the required volume by stirring gently for 1 hour with 1 g mixed-bed ion exchange resin (AG® 501-X8 (D) resin, catalog number 142-6425) per 10 ml of formamide. Filter through coarse filter paper.

For DNA or RNA Bound to Zeta-Probe Membrane (Bio-Rad Laboratories 1987)

1 mM EDTA
7% SDS
0.5 M $NaHPO_4$, pH 7.2

For DNA Bound to Nitrocellulose (Maniatis et al. 1982)

6x SSC
0.5% SDS
5x Denhardt's solution
100 µg/ml denatured salmon sperm DNA
1 mM EDTA

For RNA Bound to Nitrocellulose (Thomas 1980)

50% formamide
5x SSC
1x Denhardt's solution
50 mM $NaHPO_4$, pH 6.5
250 µg/ml denatured salmon sperm DNA

For DNA or RNA Bound to Zeta-Probe Membrane

A. Wash two times for 30–60 minutes at 65°C in:
1 mM EDTA,
40 mM $NaHPO_4$, pH 7.2
5% SDS

B. Wash two times for 30–60 minutes at 65°C in:
1 mM EDTA,
0.1x SSC, 0.1% SDS,
40 mM $NaHPO_4$, pH 7.2,
1% SDS

For DNA Bound to Nitrocellulose

A. Rinse in:
2x SSC, 0.5% SDS

B. Wash at room temperature for 5 minutes in:

For RNA Bound to Nitrocellulose

A. Wash 4 times at room temperature for 5 minutes in 2x SSC, 0.1% SDS

B. Wash two times at 50°C in:
2x SSC, O.5% SDS
1 mM EDTA

DNA Bound to Zeta-Probe Membrane

50% formamide
1.5x SSPE
1% SDS
0.5% nonfat dry milk

DNA Bound to Nitrocellulose

50% formamide
1x Denhardt's solution
0.1% SDS
100 µg/ml denatured salmon sperm DNA

Section 10
Troubleshooting Guide

I. Filtration Apparatus

1. Leakage or Cross-Well Contamination

    a. Improper assembly. The screws must be retightened under vacuum following the initial assembly.

    b. Membrane is not properly rehydrated after assembly. Always rehydrate the membrane prior to applying samples. Apply vacuum only until solutions are removed from the sample wells, then disconnect the vacuum source.

2. No Filtration or Uneven Filtration Occurring

    a. Macromolecular polymers, cellular debris, or dirt is plugging the membrane. Centrifuge samples prior to application to remove particulates. Filter solution prior to use to ensure removal of particulate material. Cover wells with Parafilm during lengthy incubations.

    b. Bubbles are obstructing the filtration. Use a needle to break any bubbles, being careful not to puncture the membrane. Pipet liquid in the wells up and down to displace bubbles.

    c. The flow valve is positioned higher than the apparatus. The flow valve must be lower than the level of the sample wells on the apparatus for proper drainage to occur.

    d. Improper blocking reagent is used. BSA is the recommended blocker for nitrocellulose; gelatin will plug the apparatus, and no filtration will occur. The Zeta-Probe® membrane, which requires more stringent blocking with BLOTTO or with gelatin and MPO, should be removed from the Bio-Dot apparatus following antigen immobilization and the rest of the assay should be conducted in a separate container.

3. Halos

    a. Membrane is not properly rehydrated before applying samples. Always rehydrate membrane prior to applying any sample.

    b. Excessive concentrations of sample are loaded. When too much sample is present, wicking into the membrane around the well will occur. Use serial dilutions of the samples to determine optimal amounts to load.

II. Poor Binding to Membrane

1. Nitrocellulose

    a. DNA/RNA will only bind efficiently in 20x SSC or 1 M ammonium acetate. Use the Zeta-Probe membrane as an alternative.

    b. DNA must be single stranded and RNA must be denatured. DNA 500 bp may not bind to nitrocellulose. Use the Zeta-Probe membrane as an alternative.

    c. Mixed-ester cellulose binds DNA, RNA, and protein very poorly. Use Bio-Rad's pure nitrocellulose.

d. Proteins 15,000 daltons may show decreased binding to 0.45 µm nitrocellulose. Use the Zeta-Probe membrane or 0.2 µm nitrocellulose. Also, glutaraldehyde fixation will increase retention of small proteins and peptides to both nitrocellulose and the Zeta-Probe membrane.

f. Protein may be removed from nitrocellulose by SDS, NP-40, or Triton X-100. Use Tween 20 in washes. Reduce concentrations or time of any SDS or NP-40 washes.

III. High Background After Incubation With Labeled Probes

1. DNA and RNA

   a. Unincorporated label, small radioactive decay products, and small probe fragments resulting from nicktranslation can increase overall background. Use the Bio-Spin® chromatography columns to remove unincorporated label. Filter hybridization solutions before use. Use the probe as soon as possible after preparation. Reduce exposure of the probe to DNase during nicktranslation.

   b. Improper blocking conditions were used. Increase the blocker concentration. Use a different heterologous nucleic acid in the prehybridization mixtures. Sonicate the solution thoroughly and denature before use.

   c. The blocker shares common sequences with host or vector of cloned probe. Vary the blocker. Yeast tRNA may be useful instead of salmon sperm DNA. Cut the probe out of vector and purify.

   d. Washes were insufficient. Include stringent washes, i.e., increase the temperature of the washes or decrease the salt concentration. Increase the number and the length of the standard washes.

   e. The probe was too hot or concentrated. Dilute the probe.

   f. The incubation period was too long. Shorten the reaction time.

   g. The bag used in hybridization collapsed on the membrane. Be sure the membrane is floating freely in the hybridization bag and that the volume of solution present is enough to prevent the bag from collapsing during incubations.

   h. Dust was present on the membrane. Remove by washing in 2x Denhardt's prior to baking or with a brief wash prior to hybridization.

   i. The gasket is contaminated by radioactivity. Replace the gasket.

2. Protein

   a. Impure secondary antibody was used. Use Bio-Rad's affinity purified blotting grade second antibody.

   b. Excessive reaction time in the substrate. Remove the blot from the substrate reaction when the signal-to-noise level is acceptable.

   c. Improper blocking conditions were used. Be sure the blocker is pure protein. Increase the blocker concentration or blocking time. Match the blocker with the detection system, Hemoglobin reacts with horseradish peroxidase; BSA may contain IgG contaminants.

   d. Primary or secondary antibody is too concentrated. Dilute the antibodies.

   e. Washes were insufficient. Increase the number and/or duration of the washes. Include progressively stronger detergents in the washes. For example, SDS>NP–40> Tween 20. Also, include Tween 20 in the antibody buffers to reduce nonspecific binding.

IV. Poor Detection Sensitivity or No Reactivity

1. DNA/RNA
    a. This problem may occur when total genomic DNA is probed for single copy or low copy number genes. Try the Zeta-Probe membrane for binding and retention of increased quantities of DNA.
    b. Hybridization was insufficient. Incorporate 10% dextran sulfate in the hybridization mixture. This polymer effectively reduces the solvent volume, thereby increasing the concentration of the solutes and enhancing hybridization.
    c. Exposure time was insufficient. Increase the time of exposure.
    d. Sample load was insufficient. Increase the sample load.
    e. Probe concentration is too low. If low signal is accompanied by low background, then the probe concentration can be increased.
    f. Binding of nucleic acid to the membrane was incomplete. See Troubleshooting Part II.
    g. If no autoradiographic signal is seen, make sure the probe was denatured by heating to 100°C, exposure to 0.4 N NaOH, or by heating to 65°C for 5 minutes in 50% formamide prior to hybridization.
2. Protein
    a. Antigen binding was incomplete. See Troubleshooting Part II.
    b. Monoclonal antibodies may not recognize a denatured antigen. Asses the binding of other monoclonal or polyclonal antibodies. Blot only native proteins.
    c. The enzyme conjugate or the substrate is inactivated. Primary or secondary antibody is inactive or nonsaturating. Test the enzyme, antibody and substrate separately for activity. Increase concentration of the primary or secondary antibody. Eliminate the detergents from reactions and washes. With HRP, avoid sodium azide, as it is a potent inhibitor of the enzyme.
    d. For labeled probes, exposure time was insufficient. Increase the time of exposure.
    e. Antibody reaction times are insufficient. Increase reaction times.
    f. Sample load was insufficient. Increase the concentration of antigen applied.

V. Nonspecific or Nonquantitative Detection

1. Protein
    a. Monoclonal antibodies may react nonspecifically with SDS-denatured proteins. Compare binding of other monoclonals or polyclonal antibodies. Blot native proteins.
    b. Concentration of the primary or secondary antibody is excessive. Increase the dilution of the antibodies.
    c. Primary or secondary antibody is contaminated with nonspecific or speciescross-reactive IgG. Use a purified IgG primary antibody fraction and affinity purified blotting grade secondary antibody.
    d. Slow, gentle filtration is needed for complete optimal protein binding.
2. DNA/RNA
    a. Probe is not pure.
    b. Blocker shares common sequences with the probe. Assess different blockers. Use more stringent washes.

Section 11
Applications and References

12.1 Common Applications

Protein

Radioimmunoassay (RIA), enzyme-linked immunoassay (EIA), fluoroimmunoassay (FIA) of soluble or particulate cellular antigens (Cleveland et al. 1981, Shen et al. 1980)

Analysis of enzymes (Faulstich et al. 1974, Huet et al. 1982)

Hormone-receptor assays (Gershoni and Palade 1983, Schafer et al. 1974)

Immunoglobulin detection (Herbrink et al. 1982, Wang et al. 1980)

Hybridoma screening (Bennett and Yeoman 1983, Hawkes et al. 1982, Horejsi and Hilgert 1983, Kane et al. 1982, Locker and Motta 1983, Shen et al. 1980)

DNA-binding proteins (Achberger and Whiteley 1981, Allen and Parsons 1979, Karagyozov and Hadjiolov 1982, Lin and Riggs 1975, Lye and Birge 1981)

Glycoprotein, lectin assays (Gershoni and Palade 1983, Neuhoff et al. 1981)

Viral antigen analysis, clinical applications (Cleveland et al. 1981, Richman et al. 1981, 1982)

Column or gel-column fraction monitoring (Cunningham 1983, Palfree and Elliott 1982, Shen et al. 1980)

Antibody purification (Olmsted 1981)

Total protein microassay (Nakamura et al. 1985)

Nucleic Acid

Dot hybridization of DNA/RNA for sequence homology, sequence abundance, etc. (Kaftos et al. 1979, Thomas 1980)

Antibody detection of DNA (Tron et al. 1983)

Viral DNA sequence detection (Berg et al. 1986, Brandsma and Miller 1980)

Hybridization selection (Harpold et al. 1978, Ricciardi et al. 1979)

Gene product and clone selection (Kranz and Gennis 1982)

Plasmid analysis (Bresser and Gillespie 1983)

CsCl, gel-column, or sucrose-gradient fraction (Cunningham 1983, Palfree and Elliott 1982, Shen et al. 1980)

DNA filter-binding assays, including DNA-drug, DNA-protein, virus-host, DNA synthesis, etc. (Bresser and Gillespie 1983)

DNA/RNA purification (Chen and Thomas 1980, Holland and Wangh 1983, Kutateladze et al. 1979, Winberg and Hammarskjold 1980)

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Section 12
Ordering Information

Catalog #	Description
170–6545	**Bio-Dot Microfiltration Apparatus**, includes Bio-Dot sample template, vacuum manifold **base plate, membrane support and gasket**
170–6547	**Bio-Dot Module**, includes Bio-Dot sample template, membrane support and gasket
170–6546	**Bio-Dot Gaskets**, 3 gaskets per package
170–6542	**Bio-Dot SF Microfiltration Apparatus**, includes Bio-Dot SF sample template, vacuum manifold base plate, membrane support, gasket, and filter paper
170–6543	**Bio-Dot SF Module**, includes Bio-Dot SF sample template, membrane support, gasket, one conversion of the Bio-Dot apparatus to the Bio-Dot SF apparatus
170–6544	**Bio-Dot SF Gaskets**, 2 gaskets per package
162–0161	**Bio-Dot SF Filter Paper**, 60 sheets
162–0117	**Nitrocellulose Membrane**, (0.45 µm), for Bio-Dot SF applications, 9 x 12 cm sheets, 10
162–0153	**Zeta-Probe Membrane**, for use with the Bio-Dot apparatus and Bio-Dot SF apparatus, 9 x 12 cm sheets, 15
732-6000	**Bio-Spin 6 columns**
732-6004	**Bio-Spin 30 columns**
142-6425	**AG® 501-X8 (D) resin,** H^+ + OH^-, 20–50 mesh, 500 g

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France 01 47 95 69 65 **Germany** 089 31 884 0 **Greece** 30 210 9532 220 **Hong Kong** 852 2789 3300 **Hungary** 36 1 459 6100 **India** 91 124 4029300
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