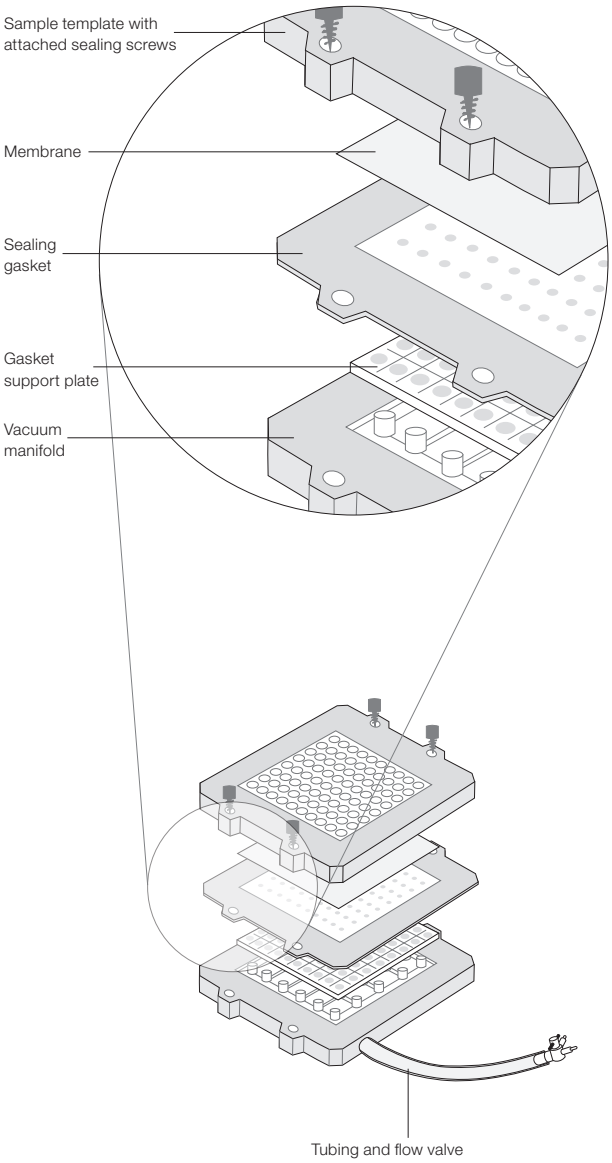
Microfiltration

Protocol

Bulletin 6215

Microfiltration



1. Prepare the samples. For best results, filter or centrifuge samples to remove particulate matter that might restrict the flow of solutions through the membrane.
2. Assemble the unit as shown in the illustration at left.
3. Adjust the flow valve so the unit is exposed only to atmospheric pressure. Add samples. Remove any air bubbles trapped in the wells by gently pipetting the solution up and down.
4. For best sample binding, the entire sample should be filtered by gravity flow without vacuuming.
5. The membrane may be washed by adding a volume of buffer equal to the sample volume in each well.
6. After application of sample, the membrane may be blocked and then probed for the protein of interest. Refer to the product instruction manual for detailed instructions.
7. To remove the membrane, leave the vacuum on while loosening the screws and removing the sample template. Then turn off the vacuum and remove the membrane.

TIPS

Application of the Vacuum
During the assay, do not leave the unit with the vacuum on. This may dehydrate the membrane and may cause halos around the wells.

Flow Valve
Proper positioning of the flow valve relative to the level of the apparatus is important for proper drainage. The speed of drainage is determined by the difference in hydrostatic pressure between the fluid in the sample wells and the opening of the flow valve that is exposed to the atmosphere. When the flow valve is positioned below the sample wells, proper drainage may be achieved.

If a prolonged or overnight incubation is desired, adjust the flow valve so that the vacuum manifold is closed off from both the vacuum source and atmosphere before applying the samples. In this configuration, solutions will remain in the sample wells with less than a 10% loss of volume during extended incubations.

To apply a gentle vacuum to the apparatus, adjust the flow valve so that it is open to the atmosphere, the vacuum source, and the vacuum manifold while the vacuum is on. Then, use a finger to cover the valve port that is exposed to the atmosphere. The pressure of your finger on the valve will regulate the amount of vacuum reaching the manifold.

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Troubleshooting

Microfiltration

Problem	Cause	Solution
Leakage or cross-well contamination	The instrument was assembled incorrectly	• Retighten the screws under vacuum following initial assembly to form a proper seal
	The membrane was not rehydrated after assembly	• Rehydrate the membrane prior to loading samples • Apply vacuum only until solutions are removed from the sample wells, then disconnect the vacuum
Uneven or no filtration	The membrane became clogged with particulates	• Centrifuge samples or filter solutions prior to application to remove particulates
	The flow valve was positioned higher than the apparatus	• Position the flow valve lower than the level of the sample wells or drainage will not occur
	Bubbles obstructed the flow of liquid	• Use a needle to carefully break any bubbles, being careful not to puncture the membrane • Pipet liquid up and down to dislodge the bubbles
	Improper blocking or antibody buffers were used	• Gelatin clogs the membrane; substitute BSA or Tween 20 for gelatin in the detection procedure
	Fluid pressure was not uniform	• Seal off unused wells or add solution to unused wells
Halos around the wells	The membrane was not rehydrated after assembly	• Rehydrate the membrane prior to loading samples • Apply vacuum only until solutions are removed from the sample wells, then disconnect the vacuum
	Too much protein was loaded, overloading the capacity of the membrane	• Determine optimum loading conditions by analyzing serial dilutions of samples
	The blocking step was too short	• Use a blocking step that is as long as the longest incubation period
	Loading volume was too low	• The meniscus contacted the center of the well, causing uneven distribution of protein sample. The minimum loading volume is 100 µl

Multiscreen Apparatus

Problem	Cause	Solution
Leakage or cross-well contamination	The instrument was assembled incorrectly	• Tighten the screws using a diagonal crossing pattern to ensure uniform pressure on the membrane surface. Do not overtighten because this will cause the channels to cut into the membrane
	The sample template has warped and can no longer provide a proper seal. (Heating the apparatus to >50°C will warp the acrylic plates.)	• Replace the sample template
Incomplete or uneven filtration	Bubbles trapped within the channels	• Tilt the instrument backward during sample application to help bubbles rise to the top • Use slow and careful delivery of reagent to prevent trapping bubbles inside the channels
Halos around the wells	The membrane was not rehydrated after assembly	• Rehydrate the membrane prior to loading samples. Apply vacuum only until solutions are removed from the sample wells, then disconnect the vacuum
	Too much protein was loaded, overloading the capacity of the membrane	• Determine optimal loading conditions by performing serial dilutions of samples
	The blocking step was too short	• Make sure blocking step is as long as the longest incubation period

This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).

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