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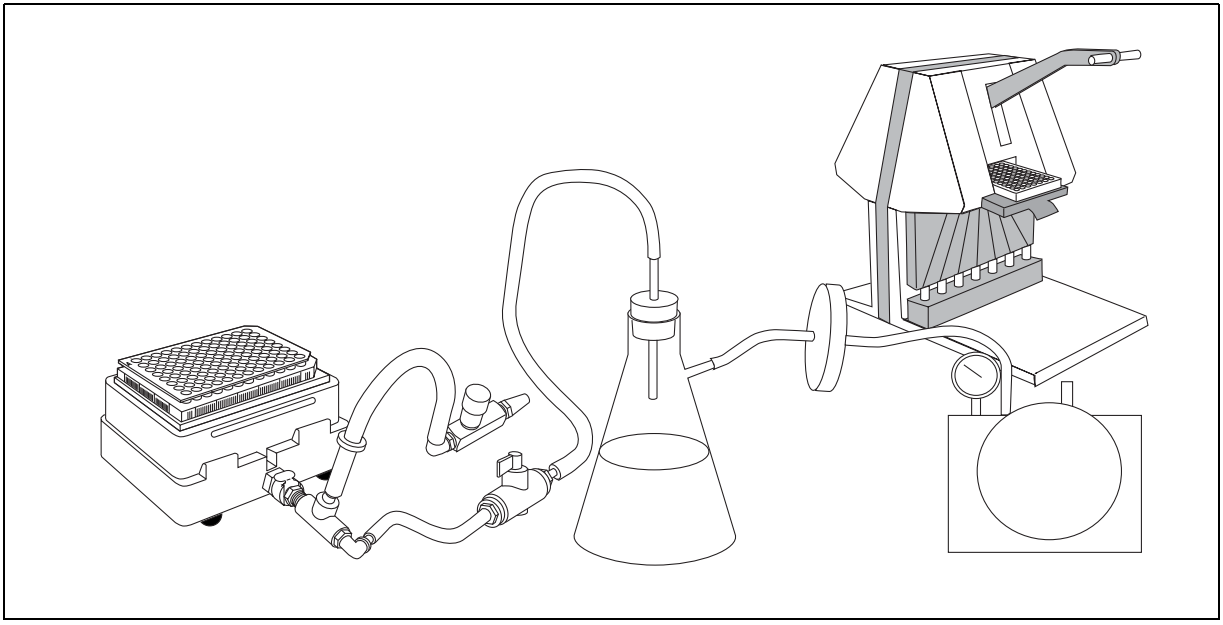
MultiScreen® Separations System User Guide



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Notice

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MultiScreen Separations System Overview

Introduction

This chapter provides information on:

- Purpose of the MultiScreen Separations System
- Function of system parts
- Use of centrifuge with MultiScreen Separations System
- Necessary additional equipment

What Is the MultiScreen Separations System?

The MultiScreen Separations System is a filtration system designed to simplify all types of separations using a 96-well format, including biochemical assays, nucleic acid purifications, high throughput sample preparation, and sample preparation for analytical instruments. You can use this system to incubate, filter, precipitate, immobilize, collect, and detect directly in the MultiScreen assay plate. The system consists of a 96-well, filter-bottomed microtitration sterile or nonsterile plate that you use with a vacuum manifold or centrifuge. MultiScreen plates are designed with individual membranes sealed to each of the 96 wells. The plate's patented underdrain enables you to do rapid and repeated washes as well as quantitative filtrate collection using vacuum or centrifuge. The underdrain support structure also allows liquid volumes to be maintained for prolonged incubation times — up to 21 days. The plate comes in a variety of microporous membranes and filter papers with various pore sizes so you can choose the right one for your application. With the MultiScreen Separations System, not only do you have a choice of porous media; you are also free to choose the most appropriate plate material as well. Choices include: *Clear* for aqueous-based applications; *Opaque* for applications requiring microplate scintillation counting or flash chemiluminescence; *Resist* for applications requiring a solvent-resistant plate; *White* for applications involving chemiluminescence or glow chemiluminescence; and *Black* for fluorescence.

How Does It Work?

Add your samples to the MultiScreen 96-well filtration plate wells, incubate, and then mount the plate on a vacuum manifold or in a centrifuge to filter. You can collect filtrate from each well into a standard or deep 96-well plate, or let wash solutions go directly to drain. After completing filtrations, you can read plates in a microplate scintillation counter or other top reading detector or use Millipore's Multiple Punch Assembly to punch the membranes from each well for further analysis using radioisotope detection. You can collect and detect the filtrate by whatever method you choose. With MultiScreen, you can process 96 samples quickly to increase your productivity without additional handling. The individual membranes in each well of the plate eliminate cross contamination concerns and enable you to obtain reproducible results. The MultiScreen system can also be used for bead-, resin-, or soft gel-based assays. Load your chromatography media into the 96 wells of the MultiScreen plate using a Millipore Column Loader and use a centrifuge designed for spinning 96-well plates to collect filtrates.

Typical Applications Using the MultiScreen System

Protein and Cell-Based Applications	Nucleic Acid Purifications	General Sample Preparation
Toxicity assays with cells and small organisms)	Dye terminator removal	96-well chromatography
Receptor/Ligand binding assays	PCR clean up	Neonatal screening sample preparation
TCA precipitation	Plasmid miniprep purifications	Combinatorial bead cleavage
Enzyme assays (e.g., kinase assays)	Prep for genomic DNA purification	Removal of bacterial contaminants
Cell culture and proliferation assays	M13 phage prep	N/A
Cell penetration assays involving mammalia, bacteria, yeast, and fungi	Reverse transcriptase	N/A
Cytokine investigations (ILs, TNFs)	In situ hybridization	N/A
Solid phase immunoassays	BAC preps	N/A
Fluorescent or chemiluminescent assays	N/A	N/A
ELISPOT	N/A	N/A

NOTE: For an up-to-date list of applications information and referenced publications, visit us on the Word Wide Web at http://www.millipore.com/multiscreen.

The MultiScreen Separations System Parts

The MultiScreen system consists of these parts:

- Disposable 96-well filtration plate assemblies
- Vacuum manifold
- Column loader for loading beads or resins
- Centrifuge alignment frames
- Adapters for automated liquid handling systems
- Plate tape for sealing plates
- Multiple punch assembly
- Eight-place carrier racks
- Disposable punch tips

The following sections provide details on the plate assemblies, vacuum manifold, and centrifugal filtration. For details on the Multiple Punch assembly, see Chapter 5.

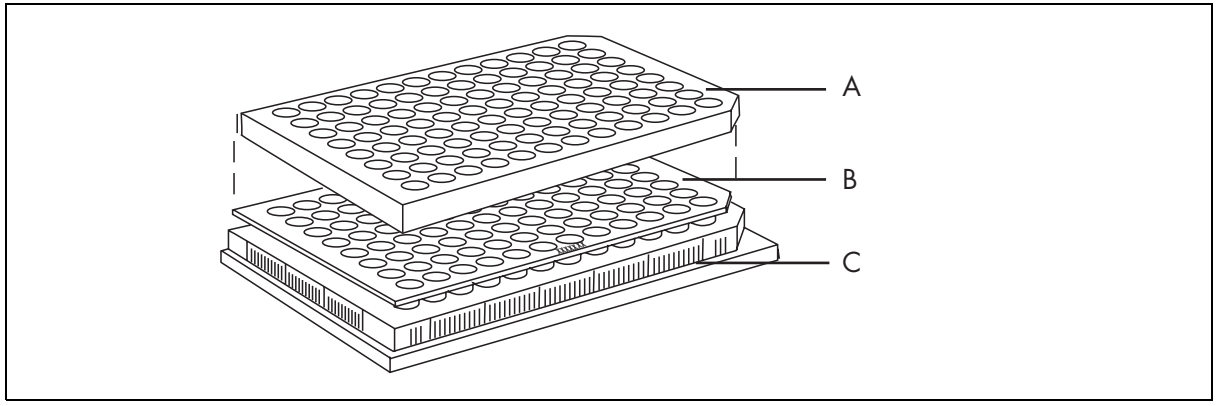
Nonsterile and Sterile MultiScreen Plates

The sterile and nonsterile MultiScreen plates consist of a cover and a 96-well plate with an integral plastic underdrain, which should be removed only ***after*** the assay is complete. If you purchased a sterile plate, it also has either a separate sterile tray or one integral to the packaging. This tray maintains the sterility of the plate bottom and may be used for incubations. Sterile plates come individually wrapped. As previously described, you can order plates with different membrane types and pore sizes, depending on your assay needs. Each plate has a catalogue number printed directly on the plate so you can easily identify the plate type.

The following section illustrates the different plates and defines the functions of the parts.

Parts and Functions of the Nonsterile MultiScreen Plate

The MultiScreen plate has three components: a removable lid (A) and a filter plate (B), which must remain integral with the underground (C).

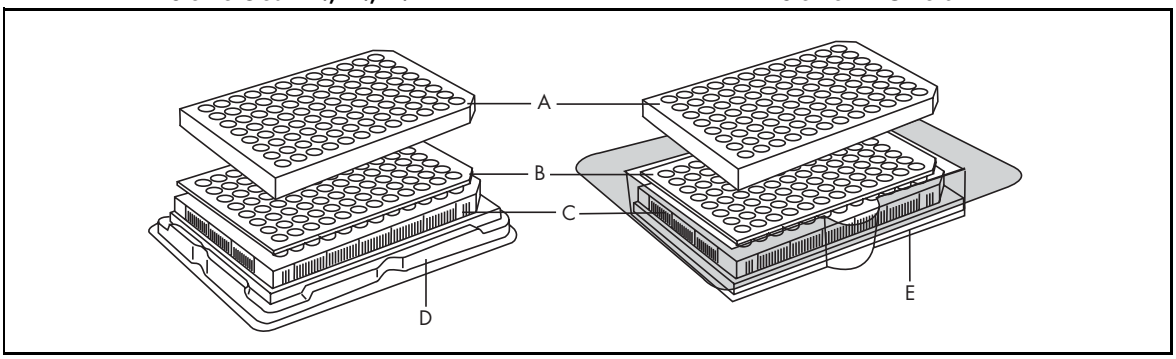


Letter	Part	Function
A	Cover	Covers the plate wells to protect them from airborne contaminants and evaporation
B/C	MultiScreen filtration plate with integral filters and underdrain (C)	■ Incubate, wash and filter to drain or to collection plate **CAUTION:** Do not remove the underdrain prior to completing your assay.

Parts and Functions of the Sterile MultiScreen Plate

Figures below show product configurations for sterile plates:

Sterile Clear HV, DV, BV **Sterile All Others**



Letter	Part	Function
A	Cover	Covers the plate wells to protect them from airborne contaminants and evaporation
B/C	MultiScreen filtration plate with integral filters and underdrain (C)	■ Incubate, wash and filter to drain or to collection plate **CAUTION:** Do not remove the underdrain prior to completing your assay.
D or E	Sterile trays	■ Maintains the sterility of the plate bottom ■ Provides consistent handling

MultiScreen Nylon Mesh Plates with 96-Well Trays

Each of these plates consists of a nylon mesh membrane on a clear, non-fluorescing, acrylic polymer plate, packaged in a MutliScreen 96-well tray. The plates are non-sterile and include lids. Each tray consists of 96 independent wells that fit the 96 wells of the membrane plate. The patented “tear drop” structure prevents air entrapment under the membrane when the MultiScreen plate is placed into the tray. The nylon mesh is not removable from the plate. The tray has optical clarity sufficient for bright field light microscopy through the bottom of the wells, but not between the wells. The available sizes are as follows:

MANM N11 50 11µm	**MANM N20 50 20µm**
MANM N40 50 40µm	**MANM N60 50 60µm**

NOTE: The plates are not suitable for use with strong solvents or prolonged exposure to concentrated alcohol. See the product insert supplied with your Millipore MultiScreen Nylon Mesh Plate for more information.

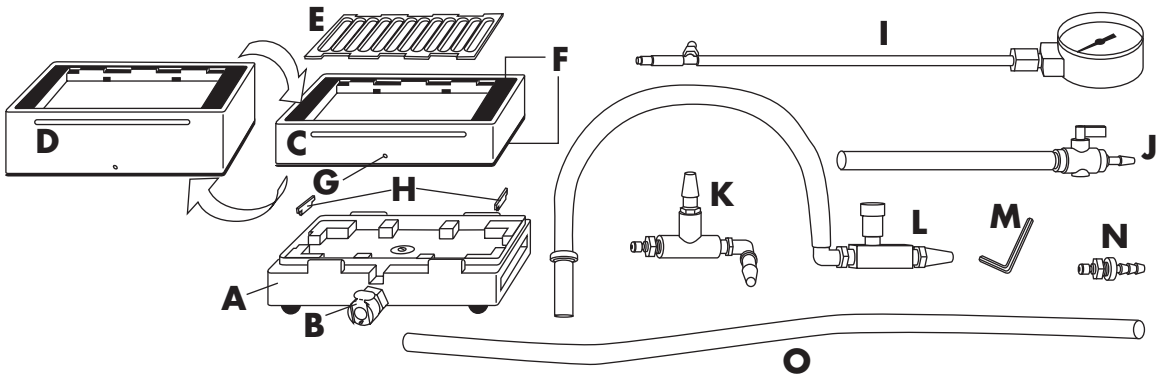
MultiScreen Vacuum Manifold

Millipore's MultiScreen Vacuum Manifold is designed for use with MultiScreen filter plates and allows rapid washing and/or collection of samples for a large number of applications. Constructed of high-density polyethylene with polypropylene fittings, this fully solvent-resistant vacuum manifold connects, preferably, to a Millipore vacuum pump or, alternatively, to "house" vacuum. The manifold's external on/off valve, vacuum control valve, and vacuum pressure gauge allow you to reliably set and measure vacuum force on filter plates. The vacuum manifold ring is sealed top and bottom with solvent-resistant EPDM gaskets. The ring comes in two sizes, standard or deep well, in order to accommodate standard or deep well (1-2 mL) receiver plates. The bleeder valve is located on the side of the ring. The stainless steel manifold support grid fits into the top opening of the manifold ring. During filtering operations, the 96-well filtration plate rests on the manifold support grid. If you need to collect the filtrate, place a receiver plate into the basin of the vacuum manifold. Some receiver plates may be slightly smaller than standard, but you can ensure that they fit snugly in the vacuum manifold basin using the plate alignment tabs.

Alternative Vacuum Systems and Adapters

Millipore's MultiScreen Vacuum Manifold is designed to allow high throughput screening assays to be incorporated into the robotics system of your choice using the optimum MultiScreen plate for the assay. Adapters are available directly from Millipore for the Beckman® Biomek® 2000 and FX units. For the most current information about robotics and robotics adapters, visit us on the Word Wide Web at http://www.millipore.com/multiscreen.

Parts and Functions of the MultiScreen Vacuum Manifold



Letter	Part	Function
A	Manifold Base	Supports standard and deep well rings
B	Quick Disconnect Body	Allows straight or 3-way connector to be attached to manifold base
C	Plastic Ring, standard well	Holds the gaskets and manifold support grid above the manifold basin to allow the use of standard receiver plates
D	Plastic Ring, deep well	Holds the gaskets and manifold support grid above the manifold basin to allow the use of deep receiver plates
E	Manifold Support Grid	Supports the plate during filtration. Must be used
F	Vacuum Manifold Gaskets	Seal the manifold support grid and ring to prevent leakage
G	Bleeder Valve	Releases the vacuum after the manifold is turned off. Must be left in ring when pressure gauge is not in use
H	Plate Alignment Tabs	Allows the correct alignment of undersized receiver plates
I	Vacuum Pressure Gauge	Allows measurement of vacuum pressure in plenum
J	On/Off Valve	Enables you to open or close the valve to the vacuum
K	Three-Way Connector	Replaces vacuum tubing connector when you want to use both the on/off valve and the vacuum control valve
L	Vacuum Control Valve	Enables you to control the amount of vacuum pressure
M	Hex Key	Use to remove or replace the bleeder valve
N	Straight Connector	Enables you to connect the manifold to your vacuum source using the on/off valve and tubing
O	FEP-lined PVC Tubing, 1/4" I.D.	Connects assemblies to vacuum pressure pump or uniform vacuum source

Centrifugal Filtration

In most cases, you will achieve faster total procedure time using the vacuum manifold rather than the centrifuge. However, some situations require that you use a swinging-bucket centrifuge with rotors for microplates. For example, gel chromatography procedures done in 96 well mini-columns usually require centrifuging in order to pack the columns. You cannot achieve optimal packing using a vacuum manifold because it results in channeling and cracking of the bed. You should also use a centrifuge when you are collecting filtrates containing high levels of solvents such as alcohols (>40%) or any surfactants and detergents such as Tween® and sodium dodecyl sulfate (SDS). Vacuum filtration of these fluids may result in mistransfer from the plate into the receiver plate. Researchers interested in using the MultiScreen plates containing VMWP membranes should use centrifugal filtration to effectively remove fluids from the wells because the pore sizes in these membranes are so small. Centrifuging can often produce results in situations where vacuum isn't effective due to plugged membrane pores.

Additional Equipment Required to Use the MultiScreen System

To use the MultiScreen plates with the vacuum manifold, you also need:

- Vacuum pressure pump or uniform vacuum source (XX55 000 00 or equivalent)

NOTE: Millipore recommends that you use a vacuum pressure pump for vacuum consistency. The pump enables you to maintain constant pressure and perform reproducible results. With a pump, you have direct connections and can use the OFF/ON switch to control the vacuum. Using another form of vacuum (house vacuum source) could cause problems, since pressure can vary depending on how much others use it and the time of day you use it in the lab.

- Millex®-FG_{50} filter (catalog number SLFG 050 10 or equivalent)

NOTE: You should use a Millex-FG_{50} filter (or equivalent) to protect the source from contamination. (See Chapter 2 for details.)

To use the MultiScreen Separations System plate with a centrifuge, you need:

- Swinging bucket centrifuge with rotors and carriers for microplates
- MultiScreen centrifuge alignment frames

For more information about MultiScreen products and accessories, you can access our web site at http://www.millipore.com/multiscreen.

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Using the MultiScreen Vacuum Manifold

Introduction

This chapter provides information on how to:

- Unpack and set up the vacuum manifold
- Operate the system using vacuum

Preparing the MultiScreen Vacuum Manifold

Before using the MultiScreen manifold, unpack the components and set them up according to the procedures outlined in this section.

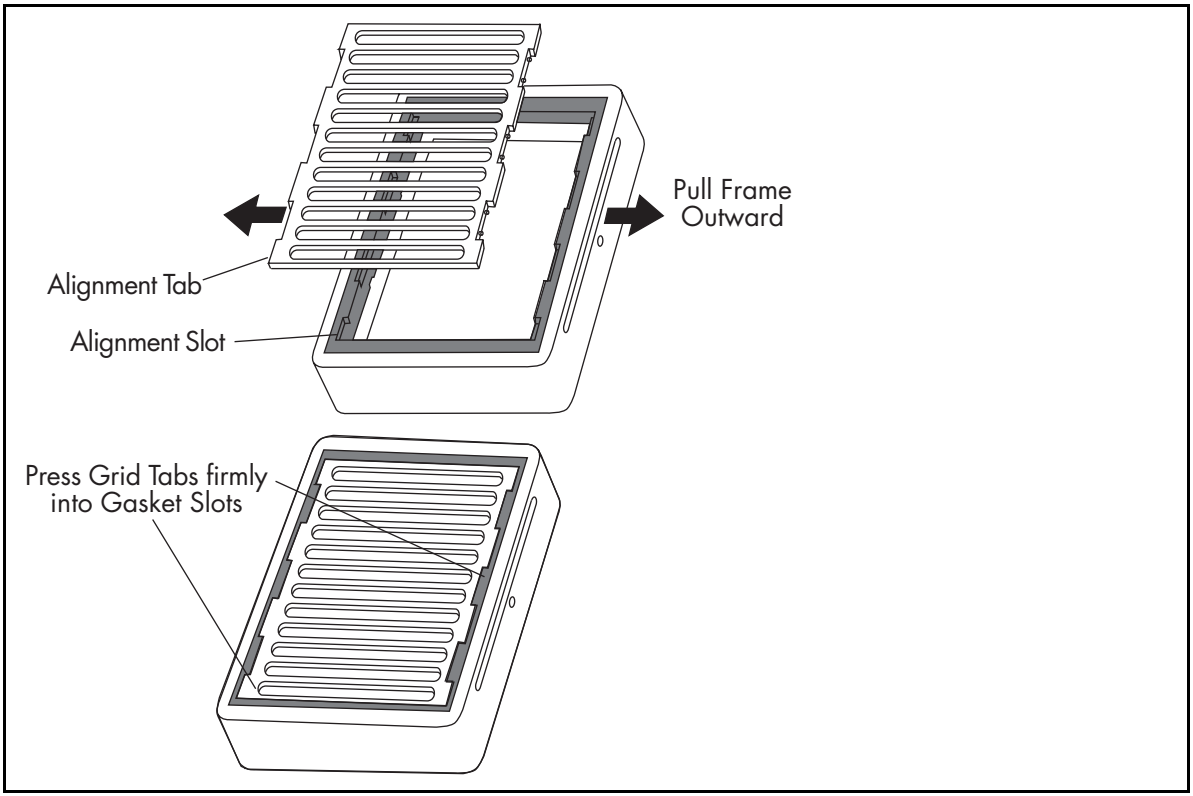
Unpacking the Vacuum Manifold

Unpack and ensure that you have the following parts of the vacuum manifold.

- Vacuum manifold base with quick disconnect body
- Standard ring assembly with gaskets, bleeder valve, and support grid
- Straight connector with quick disconnect coupling insert
- 3-way connector with quick disconnect coupling insert
- Vacuum ON/OFF valve with tubing
- Vacuum control valve with tubing
- Vacuum pressure gauge with tubing
- FEP-lined PVC tubing
- Plate alignment tabs
- Hex key wrench

Installing the Support Grid

The stainless steel support grid provides both alignment and support for the MultiScreen plate during vacuum filtration procedures. The vacuum manifold kit includes the support grid already assembled in the ring. However, if the grid becomes dislodged, you must install it in the gasket. The support grid and the top gasket have complementary tabs/slots that align the two parts properly. In addition, the grid has an elevation label stamped on one side. When correctly oriented, this label should be facing the operator when looking down at the assembled manifold. Pull out on the sides of the ring while simultaneously pressing down on the grid. When properly installed, the grid and gasket mate tightly and remain in place during routine procedures.

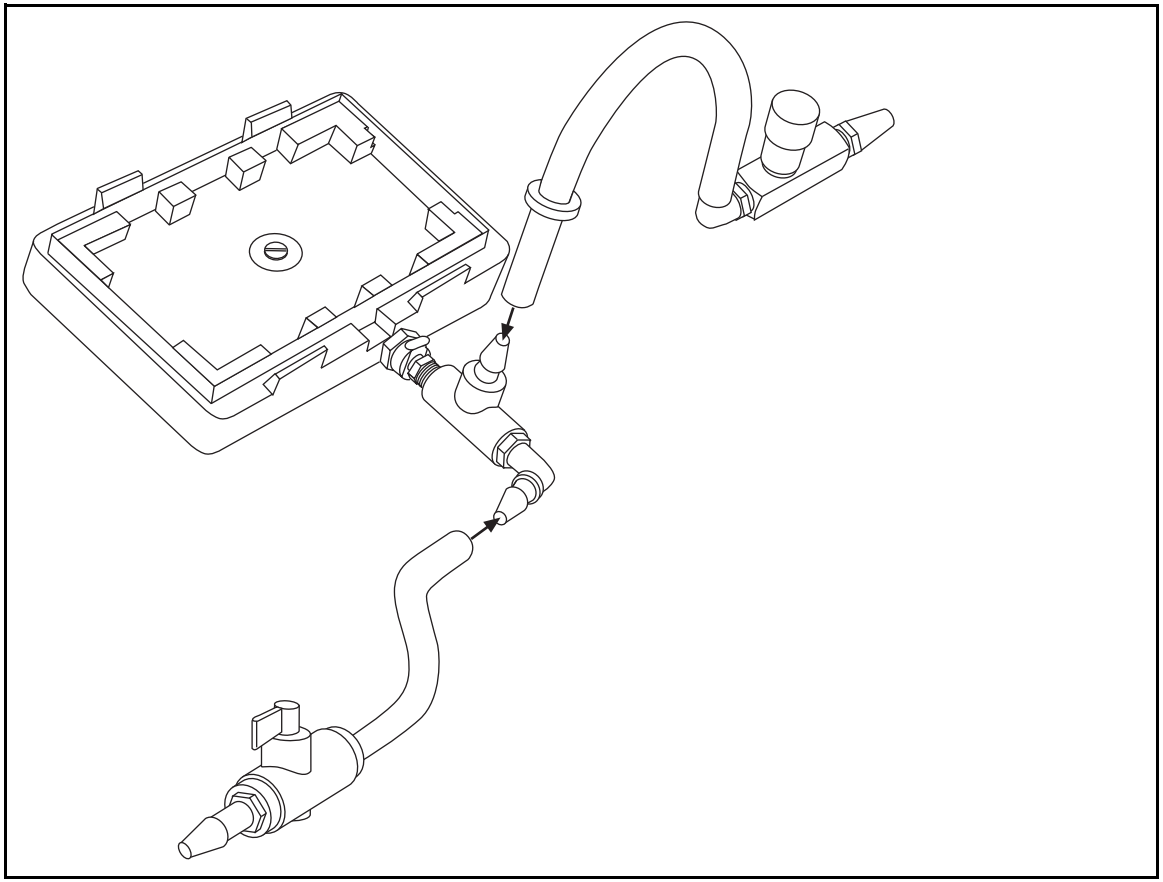


Assembling the Vacuum Manifold

1. Push the coupling insert on the end of the three-way connector into the quick disconnect body on the side of the manifold base until it clicks.

   NOTE: If you do not need to use the vacuum control valve, use the straight connector to attach the ON/OFF valve.

2. Push the end of the ON/OFF valve tubing as far as it will go onto the end fitting of the three-way connector.



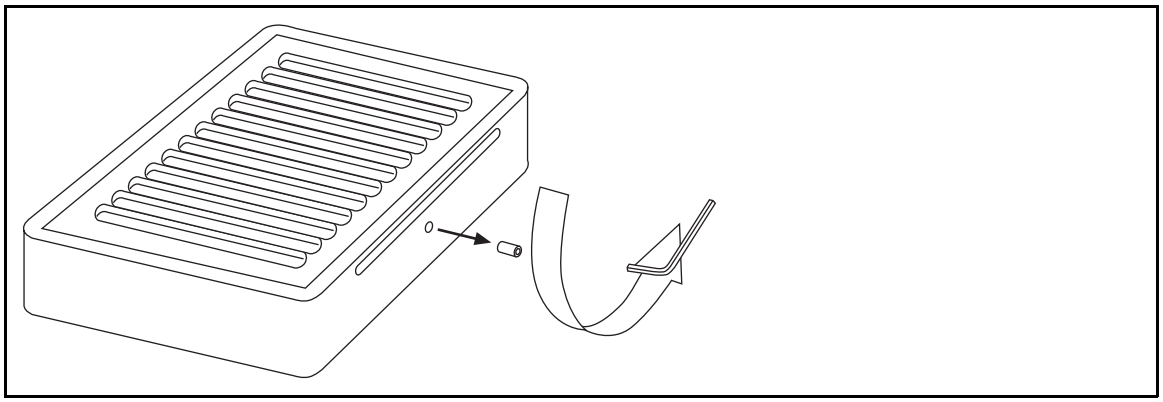
3. Push the end of the vacuum control valve tubing as far as it will go onto the top fitting of the three-way connector.

4. Place the ring assembly onto the top of the manifold base.

Installing the Vacuum Pressure Gauge

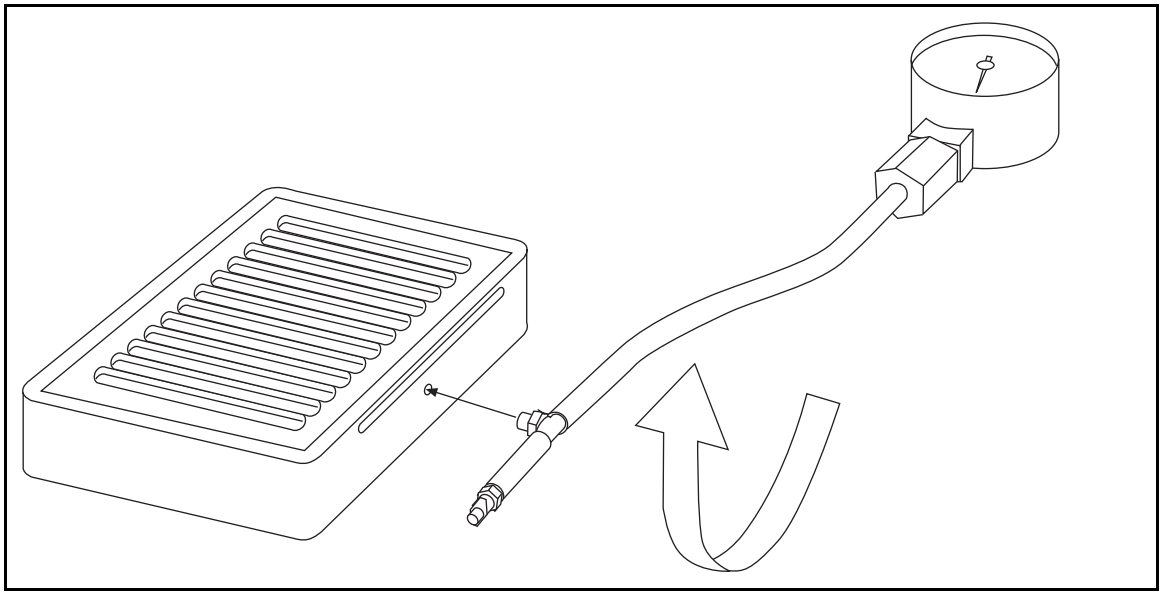
1. Remove the bleeder valve from the side of the ring using the hex key wrench provided.

   NOTE: Do not lose the bleeder valve. You will need to re-install it when you remove the vacuum pressure gauge from the manifold ring.



2. Attach the pressure gauge by screwing its connector into the ring. Use the connector that projects perpendicular to the pressure gauge tube. There is a built-in vacuum release valve at the end of the tube that hisses during manifold operation.

   NOTE: Do not overtighten the connection. No more than four rotations are required.



Assembling the MultiScreen Vacuum Manifold

1. Place the vacuum manifold on a lab bench in a stable area unaffected by vibrations from the pump (or any type of shaker).

   NOTE: Do not place vacuum pump on the same surface with the manifold.

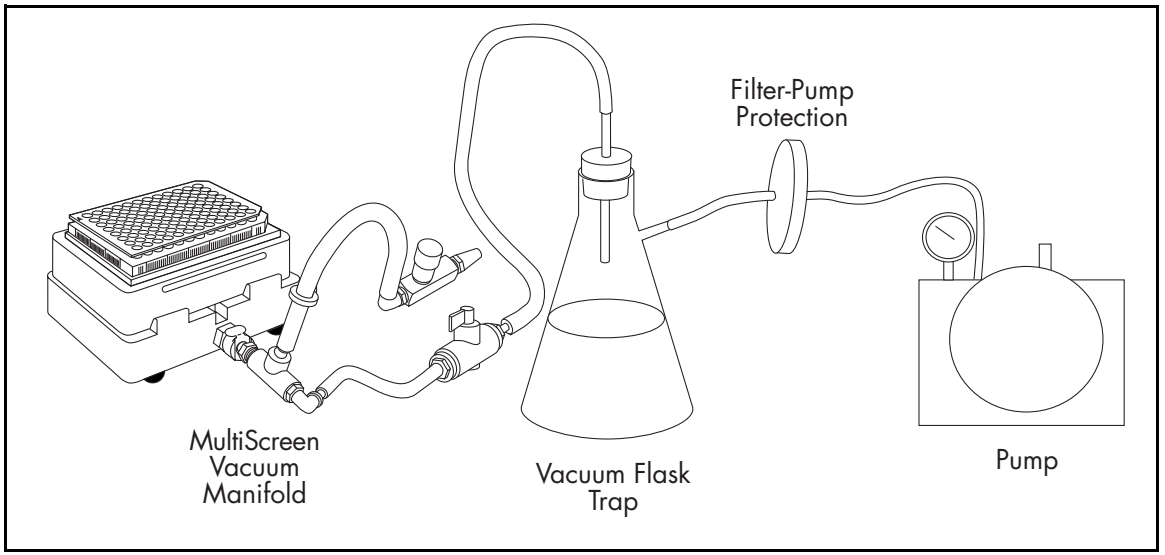
2. Place a 96-well plate in the manifold basin if you plan to do a quantitative filtrate collection.

   NOTE: If the plate you are using doesn't fit snugly in the manifold basin, see following section titled "Aligning Undersized Collection Plates."

3. Connect your laboratory vacuum source to the vacuum manifold using the tubing provided.

   NOTE: Allow plenty of room to set up the system to avoid crimping the tubing, which would reduce air flow. Crimping can also cause the lining of the tubing to crack, leading to the loss of solvent resistance. If your tubing does crimp, cut it off below the crimp and reconnect.

4. Place a Millex-FG_{50} filter and a vacuum flask trap in the vacuum line to protect the vacuum source from contamination. Your configuration should look like this:



CAUTION: Because it is possible for the Millex-FG_{50} filter to be wetted out when you are filtering organic solvents, you must use a second vacuum trap to protect the pump.

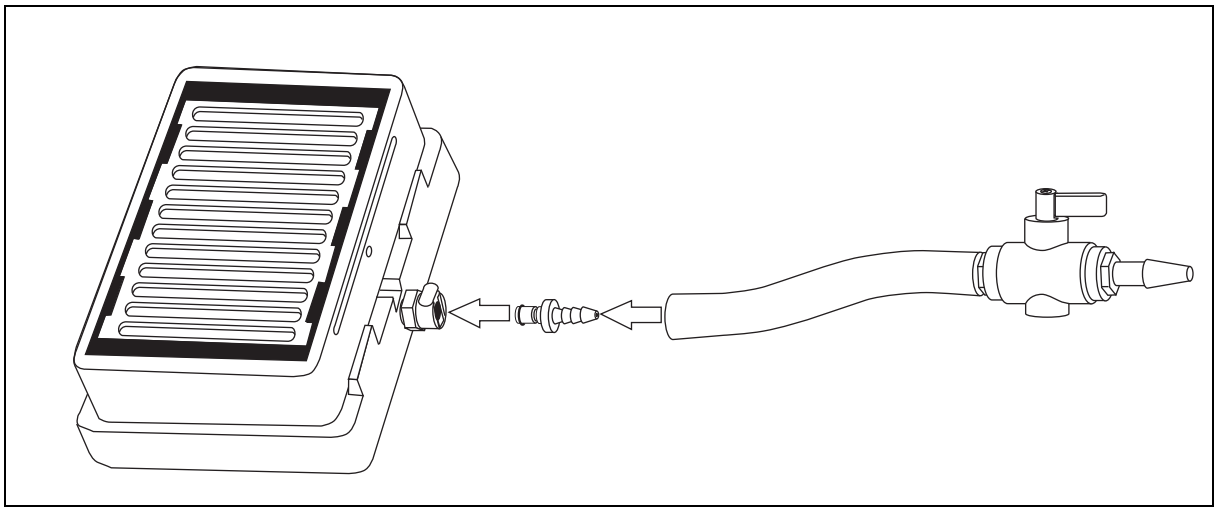
Alternative Configuration for External Control of Manifold

The current version of the vacuum manifold has a built-in quick-disconnect socket that can accept either a straight connector or a three-way connector. Both connectors are shipped with the MultiScreen manifold. Use the straight connector when your application calls only for the ON/OFF switch. Use the three-way connector when your application calls for both the vacuum control valve and the ON/OFF switch. (See the diagram in the section titled "Assembling the MultiScreen Vacuum Manifold.")

To use the manifold in the basic configuration, with the straight connector and the on/off switch, take the following steps.

1. Ensure that the three-way connector is uncoupled from the quick disconnect socket on the manifold base.

2. Push the straight connector into the quick disconnect socket until it clicks.

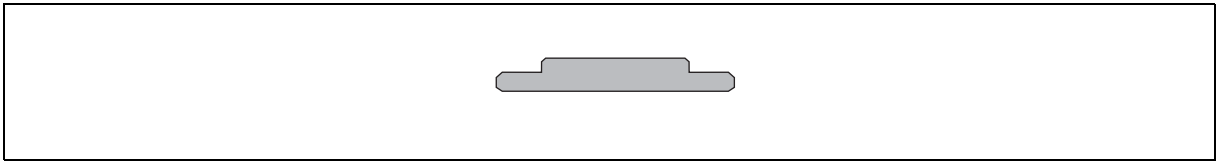


3. Push the end of the ON/OFF valve tubing onto the straight connector.

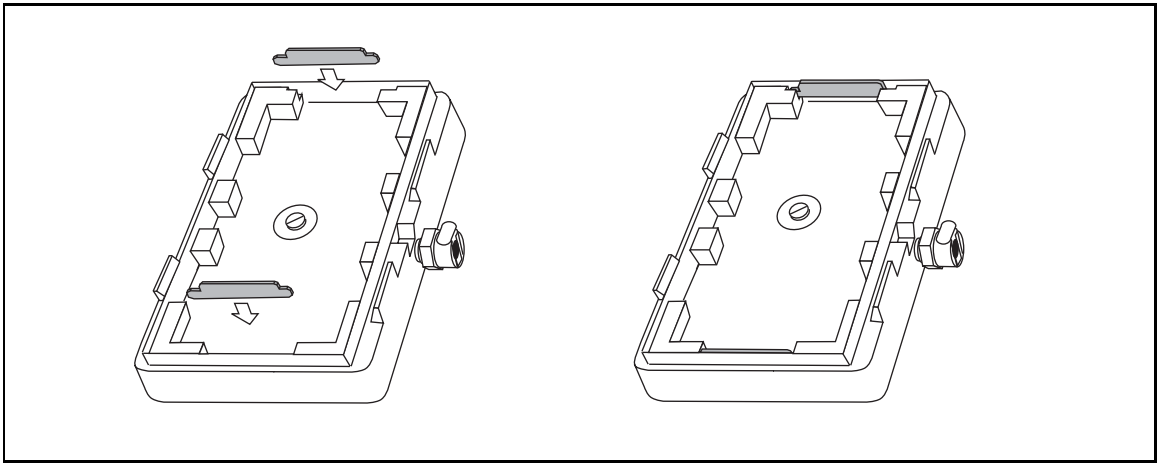
Aligning Undersized Collection Plates

You may encounter some undersized collection plates (e.g., polypropylene) and need to ensure that they will line up correctly with the filtration plates. The MultiScreen vacuum manifold comes with two plate alignment tabs that you can install when you are using an undersized collection plate. These tabs align the wells of your filtration plate with the wells of your collection plate. To install the plate alignment tabs, follow these steps:

1. Remove the two alignment tabs from the shipping bag.
2. Position the tabs with the shorter sides pointing up.



3. Insert the tabs in the manifold base by gently pressing them into the tab slots.

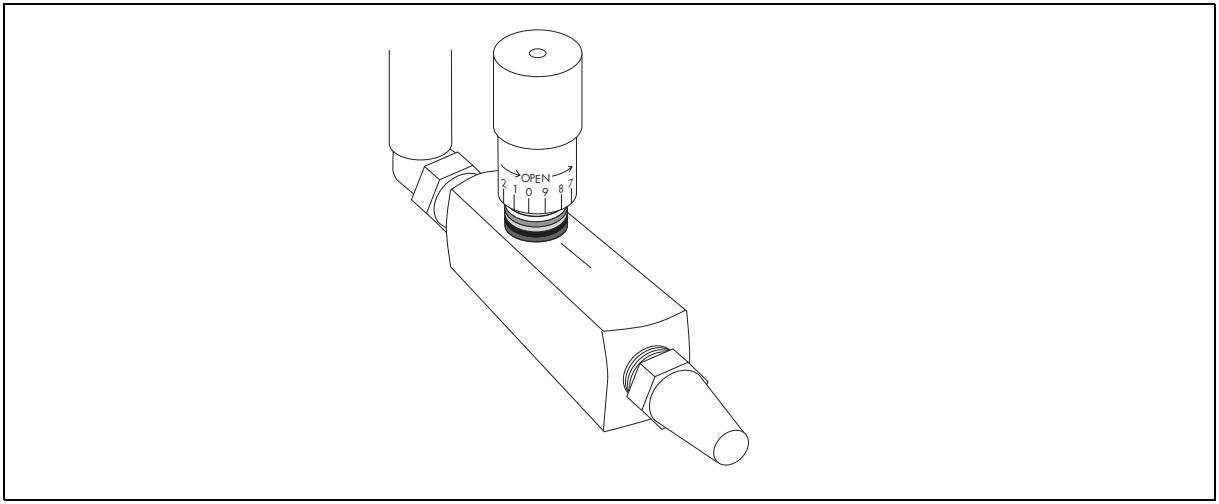


Calibrating the Vacuum Manifold

The vacuum control valve uses a system of numbers and colors to enable you to note the vacuum pressure during specific filtration procedures.

The valve is constructed with a rotating gauge on top. As you turn the gauge in a counterclockwise direction, you can align numbers on the gauge with a reference line on the body of the valve. The number facing the reference line indicates the currently-set pressure of the manifold. Additionally, a series of colors appears on the stem of the gauge as you turn the gauge. These colors assist you in aligning numbers with the reference line.

NOTE: The order of colors varies with the manufacturer of the gauge.



NOTE:Turn the gauge on top of the vacuum control valve all the way open (all colors showing) for minimum vacuum force. Close the gauge (no colors showing) for maximum vacuum force.

In order to translate these numbers and colors into more scientific terms, you may want to calibrate your manifold using the pressure gauge attached to the ring. After placing a standard (non-filter) 96-well plate on the manifold grid, turn on your vacuum source and then turn the manifold ON/OFF valve to the ON position. As you rotate the vacuum control gauge, NOTE the pressure on the vacuum pressure gauge.

Calibrating the Vacuum Manifold, continued

Make a chart like the example shown below.

NOTE: The example assumes that the series of colors is as follows: red, blue, gold, green, and silver.

Vacuum Manifold Pressure	Source Vacuum	Vacuum Control Gauge Setting
4" Hg	22" Hg	3.1 turns (i. e., setting 1 on green ring)
6" Hg	22" Hg	2.9 turns (i. e., setting 9 on gold ring)
12" Hg	22" Hg	2.1 turns (i. e., setting 1 on gold ring)
18" Hg	22" Hg	.9 turns (i. e., setting 9 on red ring)

Once you have finished calibrating your vacuum manifold, you probably want to remove the vacuum pressure gauge before beginning filtration. Unscrew the gauge from the side of the ring and **replace the bleeder valve** using the hex key wrench.

Operating the System Using Vacuum

This section provides information about preparing your MultiScreen plate for filtration using a vacuum manifold. It also includes an overview of a typical MultiScreen assay procedure and practice steps. These practice steps are not necessarily specific to the application you run with MultiScreen. For details on the exact steps you need to follow, see the assay requirements of your particular application and the MultiScreen methods for your assay. Refer to our website at http://www.millipore.com/multiscreen for technical information specific to your application.

Solvent Compatibility Information

The following table outlines the solvents that have been evaluated for compatibility with the various components of the MultiScreen vacuum manifold. Before using the manifold, verify that the solvents you intend to use are compatible.

Solvent	*Manifold Base & Tall Ring*	*Gaskets*	*Standard Ring*	*Tubing Inside: Outside*	*Support Grid*	*Control Valve*	*On/Off Valve*
Materials of Construction	HDPE	EPDM	Nylon	FEP lined Tygon	Stainless Steel	Brass Socket/ Steel Case	PP with EPDM seal
Comments				Crimping of tubing can alter resistance		Normally No Fluid Contact	
Acetone	Rinse-G	G	G-E	E:NR	E	E	G-E
Acetonitrile	E	E	E	E:NR	E	E	G-E
Dimethyl Formamide (DMF)	E	E	Rinse	E:NR	E	E	G
Dimethyl Sulfoxide (DMSO)	E	E	E	E: rinse	E	E	G
Ethyl Acetate	E	E	E	E:NR	E	G	G-E
Ethanol	E	E	G	E:E	E	E	E
Formic Acid	E	E	NR	G-E:NR	G-E	P-Rinse	G-E
Hexane	Rinse	Rinse	Rinse	Rinse-G:P	E	E	G-E
Hydrochloric Acid	E	E	Rinse -NR	E:Rinse			
Isopropanol	E	E	Rinse	E:G	E	E	E
Methanol	E	E	Rinse	E:G	E	E	E
Methylene Chloride	Rinse	Rinse	Rinse-G	E:NR	E	E	P-Rinse
Sodium Hypochlorite	E	E	NR-P	E:Rinse	G	G	G
Tetrahydrofuran (THF)	Rinse	Rinse	E	E:G	E	E	NR
Toluene	Rinse	Rinse	E	E:Rinse	E	E	Rinse
Trichloroacetic Acid (TCA)	E	E	G	E:G	G-E	Rinse	Rinse-G
Trifluoroacetic Acid (TFA)	E	E	Rinse	E:Rinse	Rinse - G	P -Rinse	G

E = Excellent performance; G = Good; Rinse = Rinse after prolonged contact; P = Rinse immediately; NR = Not recommended

Plate Preparation Requirements

Before adding assay reagents, you usually need to pre-wet the individual wells of the MultiScreen plate to ensure even reagent distribution. The pre-wet fluid you use will depend upon the properties of the membrane involved. All membranes should be pre-wet with 100 µL phosphate-buffered saline (PBS) or your first assay step buffer, except as shown in the table ("Preparation by Plate Type"). To pre-wet a plate, add the appropriate reagent, incubate for one minute, and then filter through using the MultiScreen vacuum manifold. The Immobilon™-P membrane, which is hydrophobic, should be pre-wet with 50% ethanol. The DP membrane must not be pre-wet.

Preparation by Plate Type

Plate Type	Pore Size/Membrane	Pre-Wet
GV	0.2 µm Durapore®	Yes
HV	0.45 µm Durapore	Yes
DV	0.65 µm Durapore	Yes
BV	1.2 µm Durapore	Yes
CM	0.4 µm Biopore™	Yes
R4	0.4 µm LCR	Yes
R1	1.0 µm Omnipore™	Yes
R5	5.0 µm Omnipore	Yes
DP (Protease)	0.65 µm Treated Durapore	No
IP	0.45 µm Immobilon-P	Yes (50% ethanol)
HA	0.45 µm MCE	Yes
FC, BC	Glass Fiber, Type C	Yes
FB	Glass Fiber, Type B	Yes
DE	DEAE	Yes
PH	Phosphocellulose	Yes
NA	(Proprietary)	Yes
Nylon mesh	11, 20, 40, and 60 µm	No
PCF	.4 µm black pcf	Yes
PCR	(Proprietary)	No
Plasmid	(Proprietary)	No
96-seq	(Proprietary)	No
BAC	(Proprietary)	No

Overview of Typical Operating Procedure

Although you can use the MultiScreen system for a number of different separations, these steps overview a typical MultiScreen procedure:

1. Add prepared samples (10–250 µL) to the 96-well plate wells. Cover the plate and incubate.
2. Remove the cover and place the plate on the manifold support grid.

   ▲ **WARNING: Do not operate the vacuum manifold unless the steel plate support is properly seated on the gasket to ensure proper suction and to prevent the plate from bursting away from the underdrain.**

3. Empty the plate by vacuuming the filtrate using the manifold.
4. Add reagents or samples (or both) to the plate. Cover and incubate. Repeat steps 2–4 as many times as necessary.
5. Analyze filtrate or retentate or both, depending on the needs of your assay.

Detailed Operating Procedure

This section provides steps you can follow to familiarize yourself with using the MultiScreen system. Steps in your actual application may differ. For example, you may need to incubate and run a wash before adding reagents. See the assay requirements of your application for details.

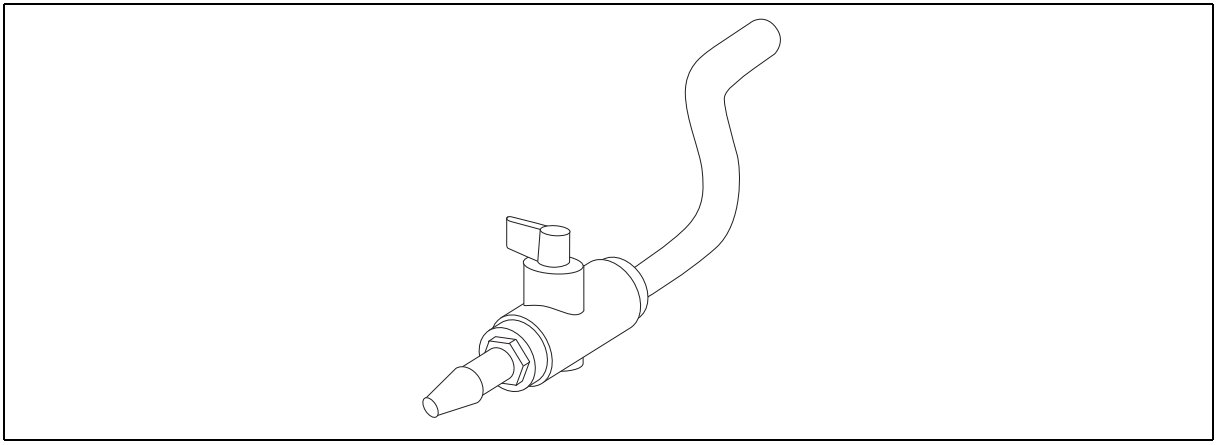
1. Remove the 96-well filtration plate from its box and place the plate at your work area on a clean surface.

   Each standard plate has a catalogue number on it for easy identification and reordering purposes. As described in Chapter 1, both the sterile and nonsterile plates include a cover and a 96-well plate with underdrain.

   CAUTION: Do not remove the plastic underdrain from any plate until the assay is completed. If you remove it before filtering samples, you have broken the plate and it will not hold fluid or work with any vacuum manifold or centrifuge. If you are performing an assay where only collected filtrate is analyzed, removing the underdrain is not necessary.

2. Prepare samples using your standard laboratory techniques. If you are using the system for the first time, you may want to prepare test samples or use plain water with an added dye to run a wash instead of using actual samples.

3. Turn the vacuum manifold ON/OFF switch to the OFF position. Set the vacuum control gauge to the minimum (all colors showing). Turn on the vacuum pump or source to evacuate any vacuum reservoirs or trap flasks.



▲ **WARNING: Do not operate the vacuum manifold unless the steel plate support is properly seated on the gasket to ensure proper suction and to prevent the plate from bursting away from the underdrain.**

Detailed Operating Procedure, continued

4. Center the plate on top of the manifold support grid and remove the plate cover. If you plan to run a quantitative filtrate collection, and you placed a 96-well plate at the bottom of the manifold, make sure the A1 well of the plate you place on top of the manifold coincides with the A1 well of the flat-bottom plate inside the manifold

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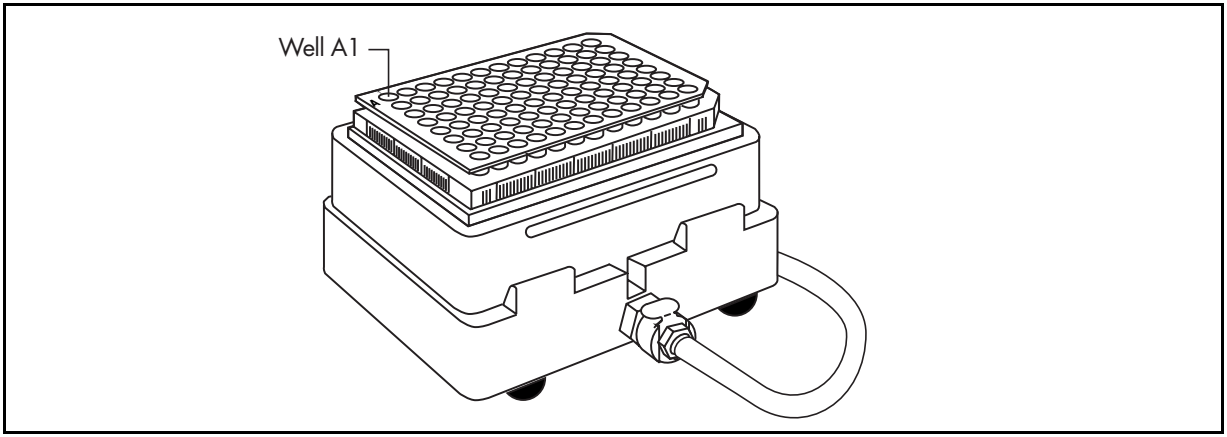


NOTE: Do not use MultiScreen plates on any manifold not equipped with the appropriate underdrain support. In addition to Millipore, several robotics system manufacturers also make approved manifolds. However, you may void the Millipore plate warranty if you do not use an approved manifold with the plates. Call Technical Service or your local Millipore office for details.)

5. Pipette the proper amount of the test sample (10 to 250 µL) into each of the 96 wells of the filtration plate.

   NOTE: If a surfactant such as Tween is present in aqueous samples or if you use a low surface tension liquid such as alcohol, it may interfere with the filtrate collection. In these cases, wash the plate with 200 µL of a surfactant-free buffer before adding the filtrate collection sample. Alternatively, consider centrifugation as a method of filtration.

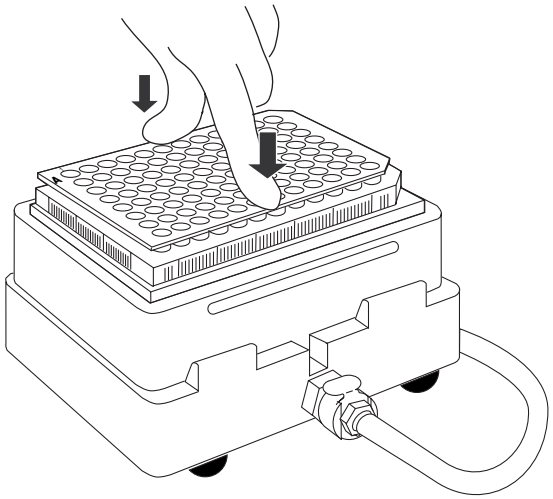
6. Adjust vacuum manifold control valve to suit your filtering requirements, which will depend on the viscosity of your fluid, the type of plate, and the pore size of the filter you are using.

   NOTE: When using DE plates, PH plates, or the Montage ^{TM} In-Gel Digest $_{96}$ Kit, always keep the vacuum flow between 4-8" Hg and turn off the vacuum source between each wash addition. To achieve this lower vacuum, you must set the vacuum pump at 12"–15" Hg and then set the manifold vacuum control valve for 4" Hg in the manifold plenum. Additionally, for certain cell types, it may be desirable to reduce the vacuum to keep the cells intact.

7. Turn the vacuum manifold ON/OFF valve to the ON position. You should see the plate attach to the manifold as the vacuum establishes the seal.

Detailed Operating Procedure, continued

NOTE: If the seal is not established, verify that the plate is centered on the manifold support grid. If this procedure is to be effective, you must have evacuated the tubing and trap flask before opening the ON/OFF valve. Some plates, such as the MultiScreen Resist plates, establish a seal with less than 12" Hg. If you are unable to use 12" Hg, help to create a vacuum seal by pressing down firmly on the center edges of the 96-well filtration plate with your fingers.



8. Observe the wells of the plate as the vacuum draws the sample through the filter material of each well and into the collection area of the vacuum manifold.

   NOTE: If the wells take more than 30 seconds to empty, increase the vacuum, unless you are using DE plates, PH plates, or the Montage ^{TM} In-Gel Digest $_{96}$ Kit.

9. Shut off the vacuum flow once the sample draws through the plate. Remove the plate assembly from the vacuum manifold and put the cover back on the plate to prevent sample contamination.

   NOTE: After shutoff, the plate requires 8–10 seconds to release naturally from the manifold. Hasten the release by opening the vacuum control valve fully (all colors showing).

3

Using a Centrifuge with MultiScreen Filtration Plates

Introduction

This chapter provides information on:

- Centrifuge equipment requirements
- How to operate the system using centrifuge
- How to perform chromatography using the MultiScreen system

Equipment Requirements

This section outlines the equipment you will need in order to perform MultiScreen separations using a centrifuge.

Centrifuge

You need a centrifuge designed for spinning 96-well plates. The following centrifuge rotors and carrier racks capable of holding two 96-well plates in each carrier have been tested for compatibility with the MultiScreen system:

- Beckman JS 4.2, JS 3.0, and JS 4.3
- IEC® 5783
- IEC 49852
- Jouan CR4-22, M4 rotor: carrier #1174168
- Sorvall® PN11065
- Beckman GH3.7 rotors
- Beckman TH-4 rotor

Microtiter Plates

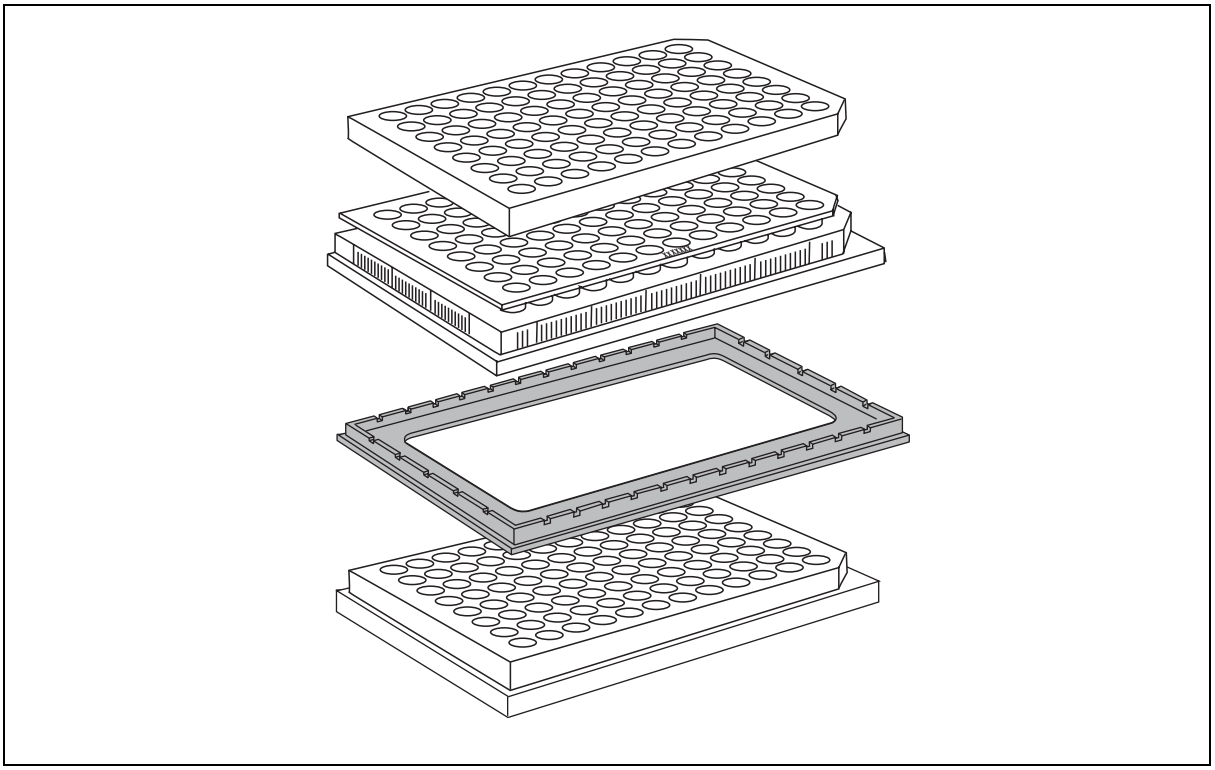
You need microtiter plates for collecting filtrate. When using rigid plastic plates, you may need to limit g-force to 1,000 g. Adding a silicone pad under the receiver plate can also limit cracking of weak plates.

Centrifuge Alignment Frames

Millipore strongly recommends that you use centrifuge alignment frames to attach the MultiScreen plate to the 96-well collection plate. Dimensions of microtiter plates, especially polypropylene ones, vary considerably. To assure accurate filtration collection into any of these plates, Millipore provides centrifuge alignment frames. These reusable units, when fitted between the receiver plate and the MultiScreen underdrain, ensure precise alignment.

Fit the centrifuge alignment frame to the top of the 96-well collection plate, then place your Multi-Screen plate on top of the frame.

NOTE: Receiver plates with no perimeter lip (for example, rimless PCR plates) do not align properly with the centrifuge alignment frame.



MultiScreen Plates

Choose the MultiScreen plate best-suited to your application. Millipore generally recommends low-binding Durapore or hydrophilic PTFE plates in centrifugal applications. For those applications that require them, Millipore produces solvent-resistant polypropylene plates with hydrophilic PTFE filters.

CAUTION: Millipore does not recommend the use of HA plates in centrifugal applications, as the membranes can crack.

Overview of Typical Operating Procedure

1. Place centrifuge alignment frame on 96-well receiver plate and fit MultiScreen plate on top of frame.
2. Add sample to wells of MultiScreen plate.
3. Put cover on MultiScreen plate.
4. Repeat steps 1–3 for two or four plate assemblies to balance centrifuge.
5. Load plate assemblies into swinging bucket centrifuge plate carriers.
6. Centrifuge.

   CAUTION: Most plates will withstand 1,000 × g, but many rigid microtiter plates fracture at 1,500 to 2,000 g. Polypropylene plates can withstand greater g-forces. Placing a thin layer of silicone rubber beneath the receiver plate can prevent the plate from cracking.

7. Separate MultiScreen plate from receiver plate and analyze filtrate, retentate, or both depending on the requirements of your application.

   CAUTION: After centrifuging, separate the centrifuge alignment frame from the underdrain by carefully inserting a fingernail or tool between them to avoid pulling off the underdrain as well.

Performing Chromatography Using the MultiScreen System

MultiScreen plates are well suited to enable 96-well processing of chromatographic media. Gel filtration media can be loaded, packed and used for the desalting and purification of proteins or nucleic acids. Additionally, loading reverse phase media with resist plates provides an excellent way to do step-wise chromatography. The cleanup and purification of samples prior to HPLC, Maldi-TOF mass spectrometry, CE and other analytical instrument-based analysis is done in high throughput mode with MultiScreen plates.

The MultiScreen Column Loader

Chromatography applications can be simplified through the use of the MultiScreen Column Loader. The Column Loader enables the quick and uniform loading of an equal volume of dry beads, gel matrix-forming powders, or resins into the 96 wells of a MultiScreen plate. The weight per well varies, depending on the media you use. The Column Loader comes in various sizes, tailored to specific applications:

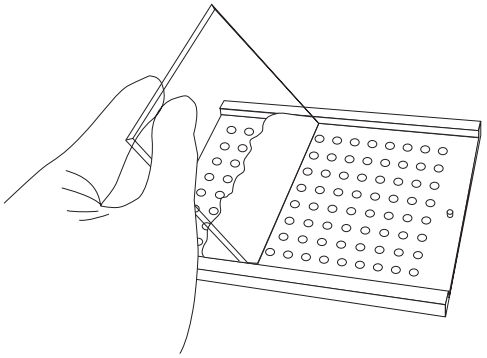
- 25 µL. Load reverse phase resins (for example, C4, C8, C18, or SCX) for sample purifications.
- 45 µL. Load small volumes of soft gels, such as Sephadex® G-50 or G- 75, for DNA or protein purification.
- 80 µL. Load Sephadex G-25 or hard resins such as Dowex® and other ion-exchange resins.
- 100 µL. Load alumina, Dowex or other resins when a high capacity is required.

Each Column Loader comes with a beveled acrylic scraper to fill the loader wells and remove the excess material back into a loading tray. The loader is made of aluminum that has been treated with a chemically inert, abrasion resistant, nonstick coating to ensure the complete transfer of material to the plates.

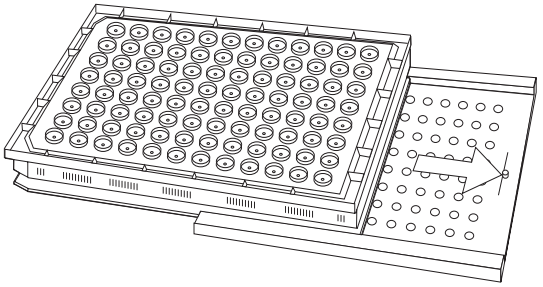
NOTE: The procedure for loading chromatography media into your MultiScreen plate using the Column Loader requires you to turn the plate upside down. Therefore, if you need to add beads after an assay has already been done in the plate, you'll need to pipette them.

How to Use the Column Loader

1. Make certain the device is clean and dry. **Do not use abrasives to clean the device.**
2. Hold the Column Loader over a suitable container, such as a plastic refrigerator container, to collect any excess resin that may spill as you pour it onto the loader surface.
3. Pour an excess of material onto the loader. Using the scraper provided, push the material into all the wells and scrape the excess off the open end and into the container for re-use.

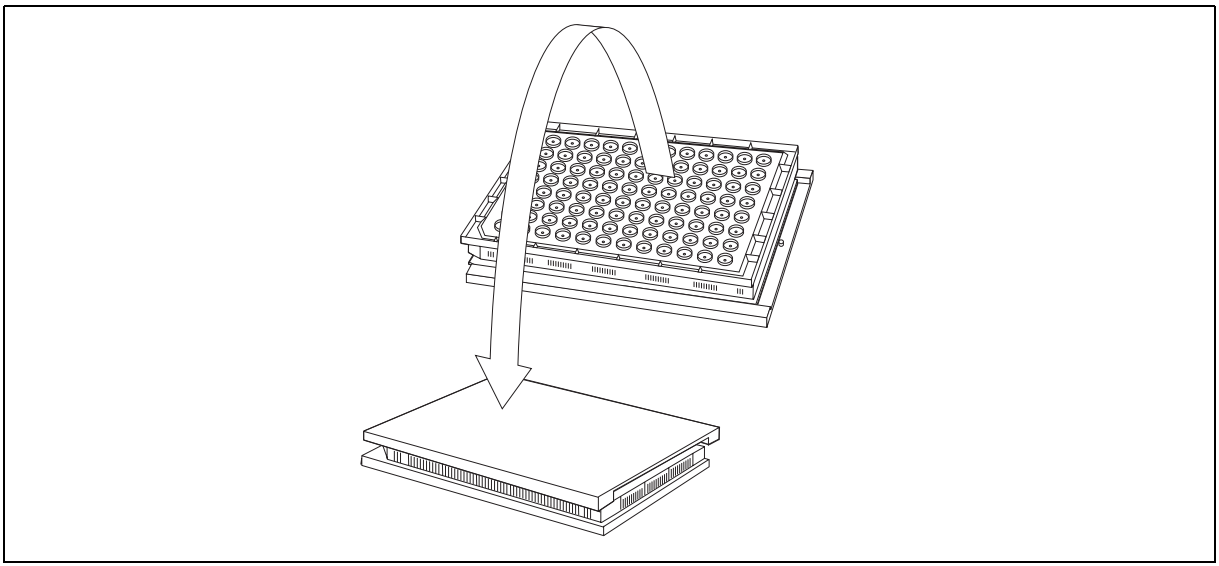


4. Slide an empty MultiScreen plate into place on the loader, upside down, so that the top of the plate is against the top of the loader. Be sure to push the plate up against the end stop to align the wells.



How to Use the Column Loader, continued

5. Hold the loader and the MultiScreen plate together and turn over the entire assembly. Tap the assembly against the side of the hood or lab bench to dislodge any particles remaining in the wells.



6. Remove the loader from the plate carefully. Your particles are now uniformly loaded into the 96 wells of the MultiScreen plate.

Gel Filtration

The MultiScreen 96-well filter plates are used for a wide variety of applications including nucleic acid purification and desalting. Using gel filtration methods similar to commercially available mini-spin columns, the MultiScreen plate offers the convenience of a 96-well format ideal for high throughput applications. The mini-columns loaded in a MultiScreen plate can handle a spectrum of sample volumes ranging from 15 µL to 100 µL and a variety of matrices. The plate provides excellent sample recovery and removal characteristics when packed with the appropriate separations media.

CAUTION: You cannot properly pack soft gel media using vacuum. Channeling and poor separations will occur. For ion-exchange media (for example, alumina, Dowex), you can use vacuum.

Reverse Phase and Ion-Exchange Chromatography

Make rapid sample preparation reproducible and cost effective by using MultiScreen plates loaded with C4 or C8 for protein purifications, or with C18 for step-wise peptide and other small molecule fractionations. Likewise, ion-exchange can be used for many protein and nucleic acid purifications.

Overview of Typical Operating Steps for Chromatography

1. Load the plates with media using the recommended column loader.

   NOTE: Gel filtration media should always be loaded over Durapore HV or R4 membrane plates. Use Resist plates for reverse phase solvents. Do not load resins or particles on top of the glass fiber, DE, or PH plates because the separations quality would be poor.

2. Pre-wet the media with 150 to 200 µL of the appropriate liquid to precondition the media, (e.g. methanol wash for C18 or proper pH and salt for regenerating ion-exchange media.)

3. Wash through and pack media as required by application.

   If you are packing the media using a vacuum manifold:

   Place the uncovered plate on the vacuum manifold, and pull through at full vacuum.

   If you are packing the media using a centrifuge:

   Fit the centrifuge alignment frame to the top of a microtiter receiver plate and place the pre-wet MultiScreen plate on top of the frame. Cover and centrifuge.

   Repeat as needed for as many plates as you plan to process.

4. Load the samples.

5. Do single or step-wise washes and elution according to your protocol.

4

Maintaining and Troubleshooting MultiScreen Equipment

Introduction

This chapter provides information on:

- Cleaning the MultiScreen manifold
- Replacing manifold gaskets
- MultiScreen Separations System problems and solutions

Maintaining the MultiScreen Manifold

Basic preventive maintenance requires that you clean the MultiScreen manifold regularly. The frequency depends on how often you use the manifold and the reagents you use. You may also have to replace the gasket in your vacuum manifold plate support occasionally. Both of these procedures are outlined in this section.

Cleaning the MultiScreen Manifold

You can use mild soap or standard laboratory detergent, bleach, or ethyl alcohol to clean all surfaces of the MultiScreen manifold. After cleaning, rinse off any residue with a soft cloth or paper towel dampened in clean water, then wipe dry. You can also use radioactive decontamination solutions and sprays.

Run a wash (buffered saline or pure water) through the system periodically to clean it. Contaminants dried in-line can change or reduce the vacuum flow over time. Make sure you fill the manifold basin to rinse it well.

▲ **WARNING: If you used the equipment for contaminated samples or radioisotopes, follow proper safety regulations when cleaning.**

Replacing the Vacuum Manifold Gaskets

If you notice the plate support on your manifold leaking, you may need to replace the EPDM gaskets as described in this section. These EPDM gaskets fit both standard and deep rings.

1. Lift the plastic ring structure (with gaskets and manifold support grid in place) up and away from the vacuum manifold base.
2. Push the manifold support grid out with your fingers.

   To remove top gasket: Pull an edge of the top gasket horizontally towards the center of the opening and upward to remove it

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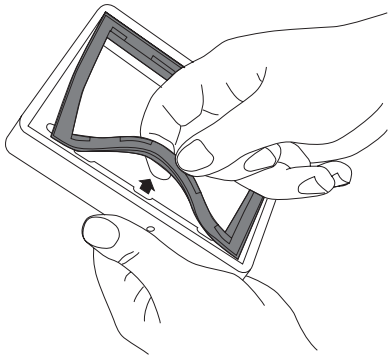
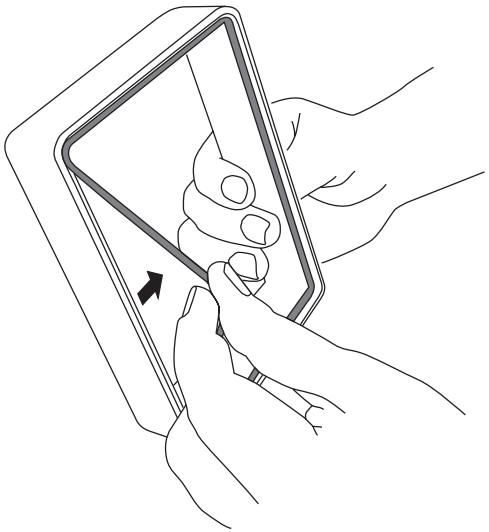


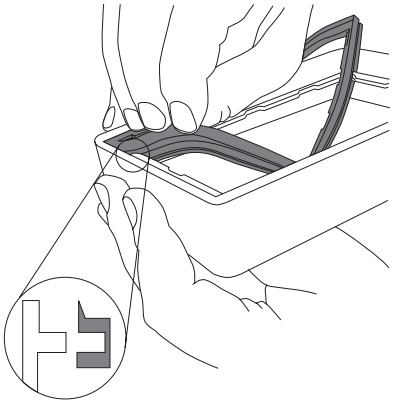
To remove bottom gasket: Gently pull up on the center of each edge of the bottom gasket to loosen it. Once all sides are loose, pull the whole thing up and off the bottom of the plastic ring.



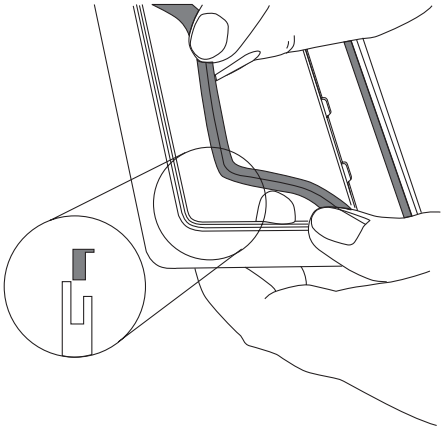
Replacing the Vacuum Manifold Gaskets, continued

3. Take your new EPDM gaskets out of their package.

   To replace top gasket: Push the groove in each side of a new top gasket onto the inside edge of the plastic ring structure. The slots that correspond to the outline of the manifold support grid should be facing up.



   To replace bottom gasket: Press the gasket into the groove inside the bottom of the plastic ring.



4. Press the manifold support grid back into the gasket, aligning its tabs with the gaskets' corresponding slots. Pull out on the sides of the ring while simultaneously pressing down on the grid, first on one side, then the other.

Troubleshooting the MultiScreen System

This section describes troubleshooting any problems you may have when using the MultiScreen system. The problems and solutions are divided into these sections:

- Vacuum manifold
- 96-well filtration plates
- Assays

Some of the solutions include literature numbers, so you can order additional information on specific applications and plates. Just call your local Millipore office and provide the listed literature number. You can also access many of the literature pieces on the Internet at www.millipore.com/multiscreen.

NOTE: If you continue to experience problems after trying some of the suggested solutions, contact Millipore Technical Service by telephone or on the World Wide Web.

MultiScreen Vacuum Manifold Problems and Solutions

Symptom	Possible Cause	Solution
No flow/ No vacuum	Lid on plate	Remove lid
	All wells not wet or unused wells not covered or sealed	Wet unused wells with Milli-Q® water, or tape the unused rows troubleshooting or columns with sealing tape. If you need to seal partial rows or columns, seal the unused rows or column with tape and leave the adjacent unused row partially sealed. Then wet out with buffer. (See Ch. 7 to order Millipore Plate Sealing Tape).
	Poor alignment of plates with gasket	Align plates
	Vacuum trap filled	Empty trap
	Filter on pump clogged	Replace filter
	Pump not turned on	Turn on pump
	Manifold on/off valve in “off” position	Turn to “on” position
	Manifold pressure gauge turned to the lowest value	Turn up to higher value
	Damaged gasket	Replace gasket (See Ch. 7 to order new gaskets)
	Bleeder valve missing (hissing sound will be heard)	Replace bleeder valve in side of manifold ring using hex key
	DE or PH plate airlocked	Reduce vacuum to 4–8 Hg. Turn off between washes (See Chapter 2, “Detailed Operating Procedure” section, step 6). Alternatively, centrifuge the plate.

MultiScreen Filtration Plates Problems and Solutions

Symptom	Possible Cause	Solution
Wells do not empty at the same time/ uneven flow	Lid on plate	Remove lid
	Vacuum line turned off or clogged	Clear line and repeat
	Samples have high particulate levels	Dilute samples in filtered buffers or switch to larger pore size, greater capacity filter (See lit. num. MM014)
	Too many cells (more than 10^6 in each well)	Dilute cell suspension (See lit. num. MM014)
	Pore size too small	Use larger pore size plate (See lit. num. MM014)
	System air locking	**If using DE or PH plates:** Shut off vacuum between each wash. Also lower vacuum to 4–8" (See lit. no. MM021) **For other plate types:** Increase the vacuum
Leakage during incubation	High surfactant concentration	Lower the concentration
	Failure to blot underdrain after filtration and before incubation	Blot underdrain
	Underdrain contacting surface	Place plate on smooth, flat surface so nothing touches the underdrain spouts
	Absorbent material contacting underdrain	Place on flat, non-absorbent material (such as a lid)
	Excessive agitation or vibration	Mix on orbital table with lower volume (maximum 200 µL on shaker, 340 µL without a shaker) or use lower speed
	Solvent evaporation sealed the lid and pressurized plate	Shim corners of MultiScreen plate so lid is higher or reduce temperature to minimize evaporation
	Organic solvents present in mixture	Lower solvent concentration

Assay Problems and Solutions

Symptom	Possible Cause	Solution
Poor replicates	Fingerprints on collection plate	Check for fingerprints on collection plate. (Don't touch bottom of underdrain.) If indicated, transfer samples to clean plate or repeat assay, taking care not to touch bottom of underdrain.
	Failure to prewet	Prewet
	Improper pipette operation or tip placement	Make adjustments
Low values on standard curve	Failure to blot underdrain after washing	Blot underdrain before punching samples
	Uneven coating of antibody	Wet membranes with plain buffer before coating with antibody
No values on samples	Check wavelength settings appropriate for substrate	Adjust accordingly
Poor transfer	Surfactant remaining in sample plate	Wash with plain buffer before adding substrate or centrifuge plate to collect filtrate
	Did not blot underdrain	Blot
	Too high level of low surface tension solvent	Decrease the concentration to $\leq$ 40% alcohol, <70% DMSO or centrifuge plate to collect filtrate
	Manifold support grid installed upside down or incorrectly	Ensure support grid and gasket mate tightly. (See "Installing the Support Grid" section in Chapter 2.)
High background	Aggregated reagents, such as the enzyme conjugate or antibodies	Use Millex filter to filter reagent before using them
	Improper membrane blocking	Add 1% BSA and 0.01% Tween 20 to diluting solutions
	Used wrong filter	Use low binding filter such as HV, DV
	Used opaque plate in glow luminescence	Use white plate or change assay detection method

MultiScreen Centrifugation Problems and Solutions

Problem	Solution
Mistransfer to receiver wells	Use Centrifuge Alignment Frame (MACF 096 04, MACF 096 S4)
Receiver plate cracks	Add silicone pad
	Reduce g-force
Poor/slow filtration	Use recommended filter: HV for Sephadex DV for Dowex R4 for Reverse Phase Media and Strong Cation Exchange

Microplate Scintillation Counting Problems and Solutions

Counter	Symptom	Solution
Packard® TopCount	Low counts	Wait ≥ 3 hours to count 3H
		Use MicroScint® 20 or 40, not MicroScint 0
		Use recommended volumes per lit. num. TN020
	Cross-talk	Use <50 µL scintillant
		Use opaque plates
Wallac MicroBeta®	Low counts	Use Supermix® or HiSafe ™ cocktail at recommended volumes
		If using DE, PH, FB, or FC plates: use recommended volumes of 25-35 µL with filter papers
	Cross-talk	Use <50 µL
		Use cross-talk correction
		Use opaque plates

5

Punching the Sample Plate

Introduction

This chapter provides information on:

- Parts and functions of the Multiple Punch assembly
- How to set up and punch the 96-well plate

Required Multiple Punch Equipment

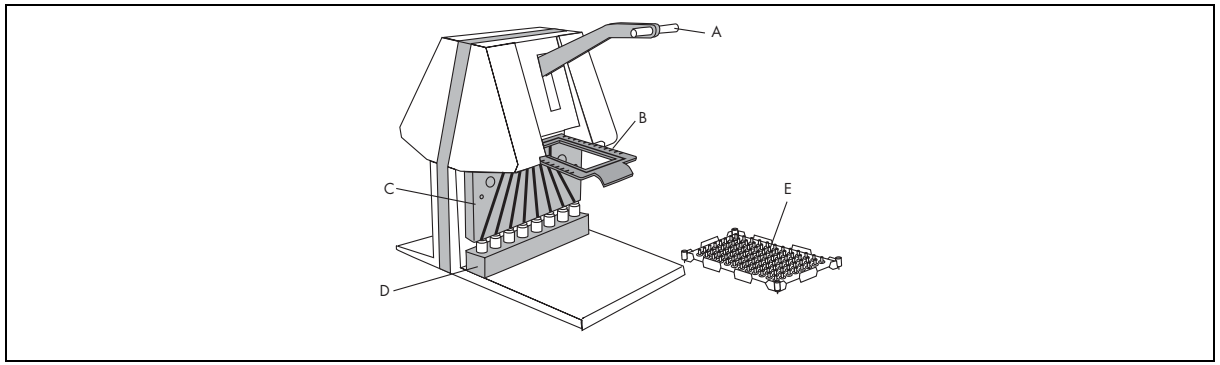
Once you complete your assay, you can punch the samples from the 96-well filtration plate. To do this, you need:

- MultiScreen Multiple Punch (includes punch carrier plate and distributor)
- Eight-place carrier rack (included in Punch Kit)
- Disposable punch tips (sold separately)

See Chapter 7 if you need ordering information.

Parts and Functions of the MultiScreen Multiple Punch

The MultiScreen Multiple Punch consists of a punch handle, a sliding punch carrier plate, a punch distributor, and an eight-place carrier rack to hold sample containers. Once you complete your assay, you can use it (with disposable punch tips) to punch membranes from each well for further analysis in scintillation or gamma radioisotope detection. The Multiple Punch looks like this:



Letter	Part	Function
A	Punch handle	Enables you to punch out the membranes
B	Sliding punch carrier plate	Aligns and secures a 96-well filtration plate to punch out the sample wells
C	Punch distributor	Distributes the sample membranes into the carrier rack containers
D	Eight-place carrier rack	Holds sample containers NOTE: The eight-place carrier racks come in different sizes to hold 4 mL vials, 7 mL vials, or 12 x 75 mm gamma tubes. (You can align and fit tubes correctly only when using the proper carrier rack.) Each rack size comes in packages of 12 with carrying tray.
E	Disposable punch tips	Spear the membranes and carry them into the carrier rack containers. NOTE: For details on the disposable punch tips, see the insert that came with them.

How to Punch a 96-Well Sample Plate

In preparing MultiScreen plates, determine whether your plate can be punched.

Evaluating the Plate Before Punching

The table below lists the various types of MultiScreen plates and states if the plate can be punched.

MultiScreen Plate	Membrane	Punchable
GV	Durapore	Yes
HV	Durapore	Yes
DV	Durapore	Yes
BV	Durapore	Yes
CM	Biopore ™	No
R4	Omnipore ™	No
R1	Omnipore	No
R5	Omnipore	No
DP (Protease)	Treated Durapore	Yes
IP	Immobilon-P™	Yes
NP	Immobilon-NC^{Pure}	No
HA	MCE	When damp
FC, BC	Glass Fiber, Type C	Yes
FB	Glass Fiber, Type B	Yes
DE	DEAE	When damp
PH	Phosphocellulose	When damp

If you determine that your plate can be punched, use the following procedure to punch the plate.

1. Place the Multiple Punch on your lab bench. Slide the punch carrier plate and distributor in and out to make sure the plate is in position.

2. Prepare your samples with the MultiScreen Separations System as described in Chapter 2 (or according to your assay). Blot the bottom of the plate on paper towels or, if necessary, sterile gauze. Peel off the underdrain of the plate.

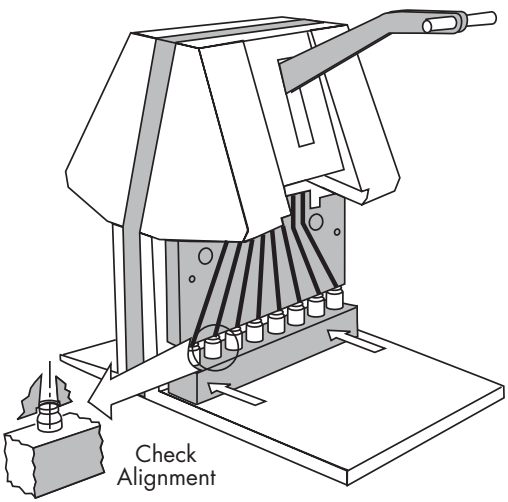
   NOTE: Once you remove the underdrain, you cannot put it back on. Therefore, you cannot punch a partial plate and use the remainder.

How to Punch a 96-Well Sample Plate, continued

3. Dry the plate under a heat lamp or other modest temperature method.

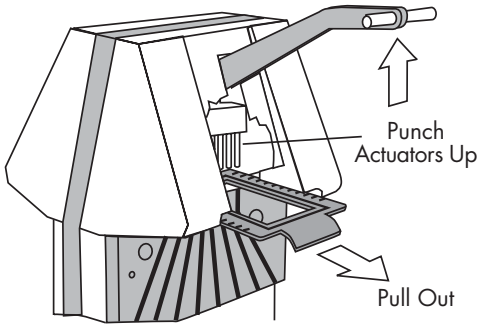
   NOTE: The 96-well plate can contain wet or dry membranes when using the Multiple Punch system depending on your assay requirements and filter type. Do not dry HA, PH or DE plates.

4. Load one to 12 separate, clean, eight-place carrier racks with your vials or test tubes (4 mL vial, 7 mL vial, or 12 × 75 mm test tube size). You may want to label the vials or test tubes to keep track of your samples. Then slide one of the racks with eight sample containers in position on the Multiple Punch base. The sample containers need to be in alignment with the punch distributor. The output of each channel should be in the center of the corresponding container. Check the alignment of the punch distributor with your sample containers each time you install an eight-place carrier rack.

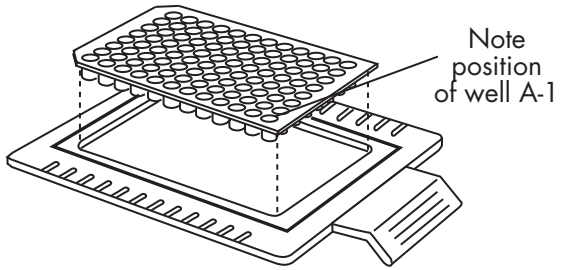


How to Punch a 96-Well Sample Plate, continued

5. Make sure the punch handle is in the up position. Pull the punch carrier plate out of the Multiple Punch assembly by pulling it forward and past all the detents (catches).

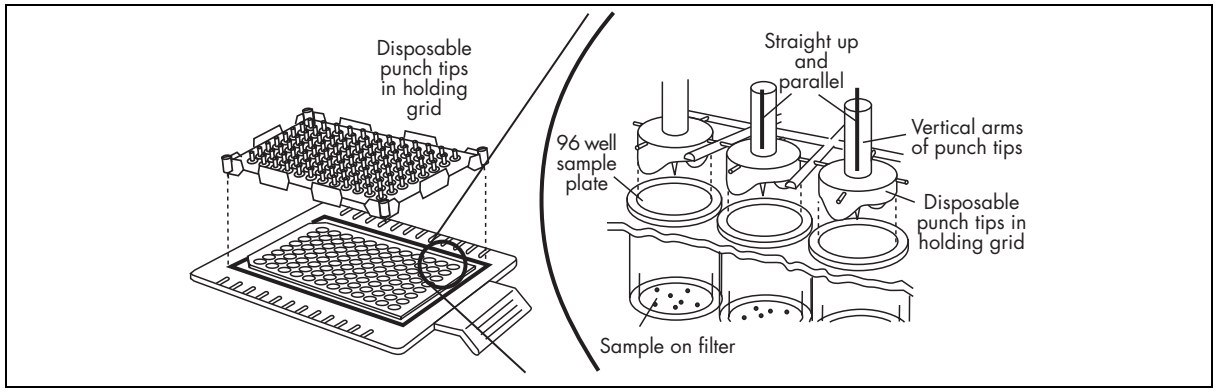


6. Place the sample 96-well filtration plate onto the carrier plate so that the wells fall within the opening of the plate. (You can only use a Millipore MultiScreen 96-well filtration plate with the Millipore Multiple Punch; plates with other dimensions do not fit.) Note that the position of the A1 well is in accordance with your sample-taking plan.



How to Punch a 96-Well Sample Plate, continued

7. Position the disposable punch tips directly over the 96 wells of the sample plate. The corner pins and side tabs fall easily into the positioning grooves on the top of the punch carrier plate. The punch tip pistons should line up above each well.

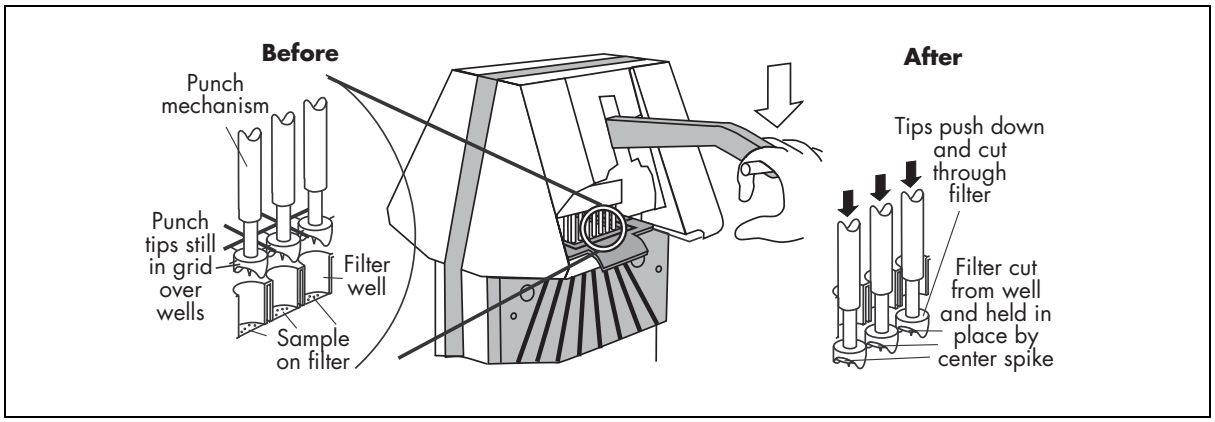


8. Slide the punch carrier plate with the loaded 96-well filtration plate back into the Multiple Punch. Gently push the carrier plate in through all the detents. You should feel a little resistance as you push the carrier plate through each detent. Once you push it until it can go no farther, pull the carrier plate out until you reach the first (innermost) detent position on the punch. (There are 12 positions.)

CAUTION: If a sample 96-well plate does not fit or slide easily into the punch assembly, make sure the vertical arms of the disposable punch tips are at the top (as shown in step 7). If necessary, reposition the tips.

How to Punch a 96-Well Sample Plate, continued

9. Push the punch handle down firmly in one smooth rapid motion. This motion causes the punch mechanism to cut through the first row of the punch tips. This motion also drives the tips through each well in the filtration plate, passing the membranes into the eight-place carrier rack containers.



CAUTION: Once you begin pushing the punch handle down, you must continue without hesitation. If you do not push it correctly, the disposable punch tips could bend, causing the membranes to puncture or burst.

10. Remove the eight-place carrier rack from the base of the multiple punch assembly by sliding it forward. Then place the next clean, eight-place carrier rack with the appropriate sample containers on the punch assembly base. Align the containers under the punch distributor.

11. Pull the carrier plate with the loaded 96-well filtration plate to the next detent position. Repeat steps 8–10 until you punch all of the samples out of your 96-well filtration plates and into containers. Once done, examine your samples as required for your procedure. (Add a liquid scintillation cocktail or directly count.)

▲ WARNING: When you complete your procedure, dispose of the samples and radioactive and chemical waste according to proper safety regulations.

6

Maintaining and Troubleshooting Multiple Punch Assembly Equipment

Introduction

This chapter provides information on:

- Cleaning the Multiple Punch assembly
- Troubleshooting common assay problems with the Multiple Punch assembly

Maintaining the Multiple Punch Assembly

This section describes how to:

- Clean the equipment
- Remove the punch distributor of the Multiple Punch assembly for cleaning, decontaminating, or adding anti-static spray

Cleaning the Multiple Punch Assembly

The Multiple Punch Assembly requires cleaning at a frequency that depends on the type of filter in question. For example, if you punch glass fiber filters, you may need to clean it weekly; however, if you punch dry Durapore membrane plate applications, you need clean the punch only rarely.

To clean the Multiple Punch assembly, you need:

- Mild soap or standard laboratory detergent
- Clean soft towels or paper wipes
- Water

NOTE: You can also use radioactive decontamination solutions and sprays.

You can clean the outside surfaces of the Multiple Punch assembly, the punch distributor of the assembly, and the eight-place carrier racks using a water-dampened soft cloth or paper towel and a mild soap or standard laboratory detergent. You can also remove the punch distributor and the punch carrier plate from the punch assembly for cleaning. (See the next section for details.)

After cleaning, rinse off the soap residue with a soft cloth or paper towel dampened in clean water, then wipe dry.

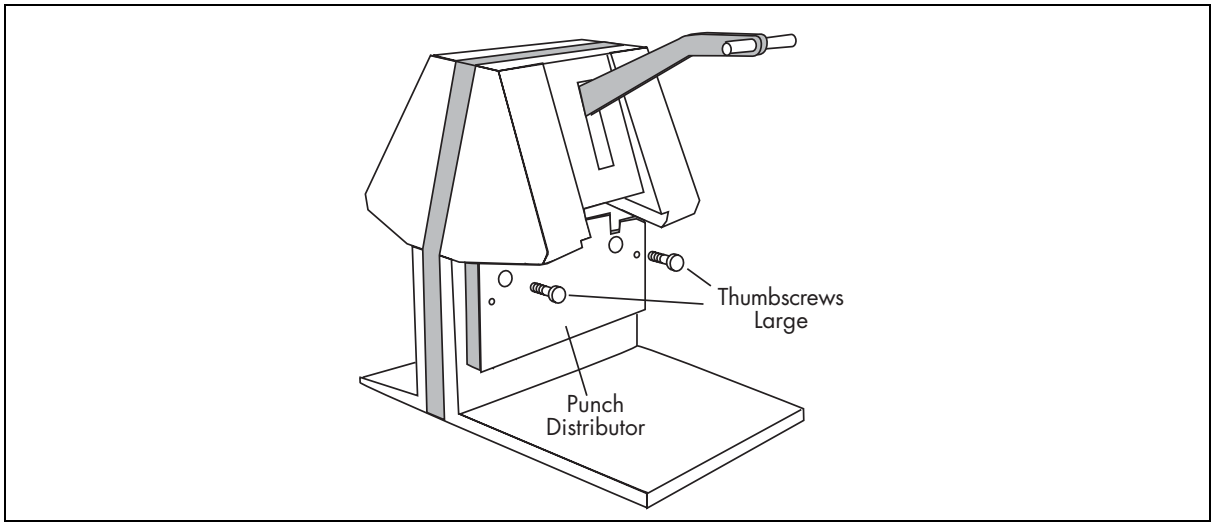
▲ **WARNING: Follow proper safety regulations when cleaning.**

Removing the Punch Distributor from the Multiple Punch Assembly

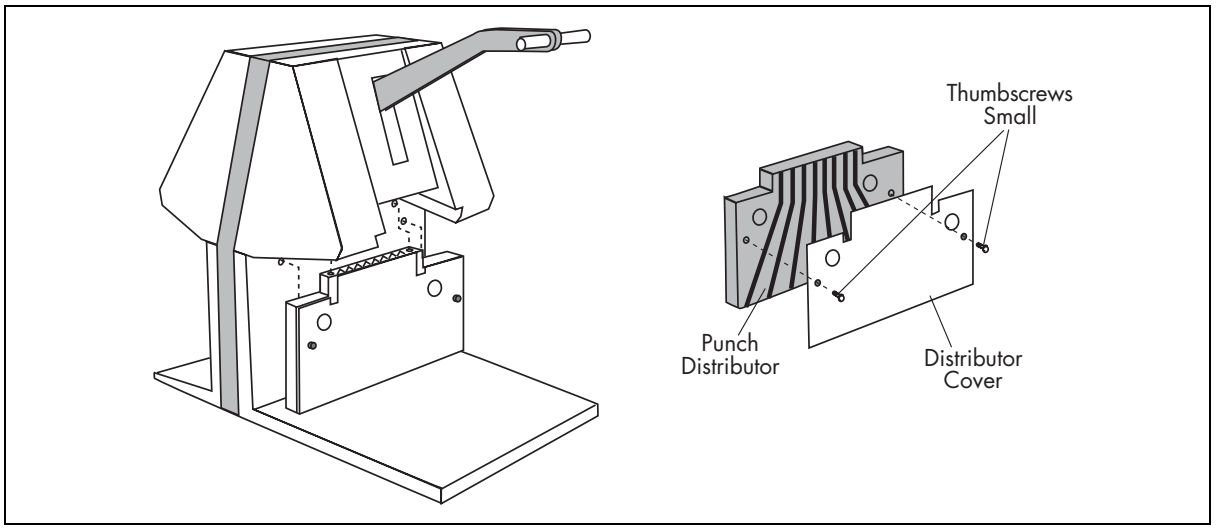
If the equipment is found to be radioisotope contaminated or if you want to perform a wet cleaning, you should remove the punch distributor from the assembly and clean it as described in the "Cleaning the Multiple Punch Assembly" section. (Use proper safety methods if the equipment is radioisotope contaminated.) You need to remove the punch distributor to decontaminate it. You must also remove the punch distributor if a sample does not pass through the punch distributor fan or otherwise becomes stuck inside the punch.

1. Remove the eight-place carrier rack from the base of the Multiple Punch assembly by pulling it straight out towards you.

2. Remove the two larger thumbscrews at the front of the Multiple Punch assembly. Hold on to the punch distributor as you remove the thumbscrews so that the distributor does not fall off the assembly.



3. Pull the punch distributor down and out from the Multiple Punch assembly. If you need to remove sample membranes or decontaminate the punch distributor, remove the punch distributor cover by removing the two smaller thumbscrews.



NOTE: If membranes remain in the punch distributor because of static electricity, use tweezers to move the sample into the proper container. To prevent other membranes from getting trapped, apply an antistatic spray to the distributor, cover, and assembly base.

4. Replace the distributor cover (if removed). Reattach the punch distributor to the assembly base by screwing it back into place once you clean or decontaminate the parts. Then slide the eight-place carrier rack back into the base of the Multiple Punch assembly.

Troubleshooting the Multiple Punch Assembly

This section describes troubleshooting any problems you may have when using the Multiple Punch assembly. The problems and solutions are divided into these sections:

- Multiple Punch assembly
- Radioisotope or detection

Some of the solutions include literature numbers so you can order additional information on specific applications and plates. Just call your local Millipore office and provide the literature number listed with the solution. You can also access many of the literature pieces on the Internet at www.millipore.com/multiscreen.

NOTE: If you continue to experience problems after trying some of the suggested solutions, see Chapter 7 for Millipore Technical Service information.

Multiple Punch Assembly Problems and Solutions

Problem	Possible Cause	Solution
Membrane remains on plate after punching	Glass fiber filters on tip, Durapore under membrane remained on plate	All counts in fiber, just some Durapore filter remains on plate (See lit. num. MM010, MM012, and MM013)
	HA membrane	Punch damp membrane only (cannot be dry)
All disks do not punch out	Disposable punch tip array bent	Check punch tips to make sure that they are vertical
	Improper Multiple Punch assembly operation	Use smooth, rapid downstroke
Membranes remain in punch distributor	Static electricity	Apply static spray (See “Removing the Punch Distributor from the Multiple Punch Assembly” section in this chapter)
	Misaligned vials or tubes	Align vials or tubes
	Punch distributor cover not aligned	Align cover

Other Radioisotope or Detection Problems and Solutions

Problem	Possible Cause	Solution
No counts for liquid scintillation	Undissolved counts (for example, tritium isotope)	Recount after 18 hours (or overnight). (See lit. num. MM010.)
Low count	Cell incorporated activity — failure to add punched membranes to bleach solution before adding cocktail	Add 500 µL of 0.42% concentration sodium hypochlorite to vial and repeat procedure. (Run appropriate bleach quench controls with your liquid scintillation cocktail.) (See lit. num. TB038.)
TCA did not precipitate protein completely	TCA not ice cold or incubation not long enough	Repeat assay with ice cold TCA solution. Incubate on ice or at 4°C for a minimum of 30 minutes. (See lit. num. MM010.)
	Failure to add water to punched membranes before adding cocktail	Add 500 µLs of water or 0.42% concentration sodium hypochlorite to the vial and repeat the procedure. (See lit. num. MM010.)
	Histone/myelin basic protein	Require 25% TCA final concentration to retain precipitate.
	Too little protein in wells	Add carrier protein to total protein >10 µg/well.
Erratic sample scintillation counting	Poor water to cocktail mixture	Mix thoroughly and count samples again after waiting for at least three hours. Add water to dissolve precipitate.
	Tritium isotope	See lit. num. MM010.
Low values for samples or standards (or both)	Cocktail volume not correct	Check volume of cocktail and water mixing capability.
	Microplate scintillation counters	Can give lower counter efficiencies, particularly for tritium 3H. (See lit. num. MM013 for optimization.)
	Quenching procedure not correct	Run appropriate quench controls.
	Counts passed through filter	Use smaller pore size membrane or glass filter. Use ice cold TCA.

7

Accessing Technical Information

Introduction

This chapter provides information on:

- Specifications of the MultiScreen system components
- Ordering information and technical service
- Warranty

Specifications

The following table lists the physical characteristics of the components of the MultiScreen Separations System. The weights are approximate.

Storage Conditions for Plates

Store the plates in a controlled environment at a temperature between 15 °C and 30 °C.

Vacuum Manifold with Standard Ring

Dimensions	H × W × D: 6.50 × 15.24 × 12.07 cm (2.56 × 6.0 × 4.75 in.)
Weight	537 g (1.18 lb)
Shipping Weight	1,956 g (4.32 lb)

Vacuum Manifold with Deep Ring

Dimensions	H × W × D: 9.37 × 15.24 × 12.07 cm (3.69 × 6.0 × 4.75 in.)
Weight	605 g (1.33 lb)

Fully Assembled Vacuum Manifold with Vacuum Control Gauge, On/Off Valve, Pressure Gauge

With Standard Ring

Dimensions	H × W × D: 16.51 × 53.34 × 22.86 cm (6.5 × 21 × 9 in.)
Weight	798 g (1.76 lb)

With Deep Ring

Dimensions	H × W × D: 16.51 × 53.34 × 22.86 cm (6.5 × 21 × 9 in.)
Weight	867 g (1.91 lb)

96-Well Filtration Plate Assembly

Dimensions	H × W × D: Nonsterile, 1.9 × 12.7 × 8.6 cm (0.75 × 5.0 × 3.4 in.) H × W × D: Sterile, 2.5 × 15.7 × 11.0 cm (1.0 × 6.2 × 4.3 in.)
Weight	Nonsterile, 0.07 kg (0.15 lb) Sterile, 0.1 kg (0.21 lb)
Shipping Weight	Box of 10, Nonsterile, 0.7 kg (1.5 lb) Box of 50, Nonsterile, 3.7 kg (8.1 lb) Box of 10, Sterile, 0.9 kg (2.0 lb)

Multiple Punch Assembly

Dimensions	H × W × D: 43.8 × 22.9 × 35.6 cm (17.3 × 9.0 × 14.0 in.)
Weight	8.1 kg (17.75 lb)
Shipping Weight	9.1 kg (20.0 lb)

Eight-Place Carrier Rack Assembly (Includes 12 Eight-Place Racks)

Dimensions	H × W × D: 4.4 × 53.9 × 25.0 cm (1.7 × 21.2 × 9.8 in.)
Weight	3.63 kg (1.65 lb)
Shipping Weight	5.83 kg (2.65 lb)

Disposable Punch Tips Assembly

Dimensions	H × W × D: 0.7 × 13.5 × 8.9 cm (0.31 × 5.3 × 3.5 in.)
Weight	0.02 kg (0.03 lb)
Shipping Weight	Box of 10, 0.2 kg (0.3 lb) Box of 50, 1.2 kg (2.5 lb)

Materials of Construction

The following table describes the materials of construction within the MultiScreen system.

Part	Area	Description
Vacuum manifold	Base	HDPE
	Standard ring	Nylon
	Deep ring	HDPE
	Support grid	Stainless steel
	Gaskets:	EPDM
	Control gauge socket	Brass
	Control gauge case	Steel
	Pressure gauge	Steel
	On/Off valve	Polypropylene with EDPM steel
	Tubing	FEP-lined Tygon®
Punch	Disposable punch tips	Polystyrene
Quick disconnect fittings	Main components and valve	Polypropylene
	Thumb latch and valve spring	Stainless steel
	O-ring	EDPM
Cell culture trays	Main components	Clear, non-fluorescing acrylic polymer
MultiScreen 96-well tray	MANM N11 50	11 µm; nonsterile, with lid
	MANM N20 50	20 µm nonsterile, with lid
	MANM N40 50	40 µm nonsterile, with lid

NOTE: These non-sterile plates are intended for applications to conduct testing of insects, nematodes, and other organisms. The nylon mesh is not removable from the plates. The trays have optical clarity sufficient for bright field light microscopy through the bottom of the wells, but not between the wells.

Recommended Plates

If you need help determining which plate would be best for your application, please contact Millipore's local Technical Service office. If you need information about ordering MultiScreen products, request literature number FF005EN00. We also have MultiScreen information available on our web site at www.millipore.com/multiscreen.

NOTE: Millipore is continually developing new plates, membranes, and applications. Call your local Millipore office for the latest information or to request what you need.

MultiScreen Filtration System Vacuum Manifold and Accessories

	Catalogue Number	Qty/Pack
Vacuum Manifold Basic Kit — Includes manifold base, standard ring with gaskets, support grid, all tubing and valves, and pressure gauge	MAVM 096 0R	
Vacuum Manifold Deep Well Ring with Gaskets	MAVM 096 0T	1/pk
Vacuum Manifold Replacement Gasket Set (1 each/top & bottom)	MAVM XXA 04	1 set
Vacuum Manifold Replacement Support Grid	MAVM XXA 05	1/pk
Vacuum Manifold Replacement Tubing Set	MAVM XXA 06	1/pk
Vacuum Manifold Replacement On/Off Valve, Vacuum Pressure Gauge, Vacuum Control Valve, and Tee Assembly	MAVM XXA 07	1/pk
Vacuum Manifold Replacement Standard Well Ring with Gaskets	MAVM XXA 08	1/pk
Vacuum/Pressure Pump, 115 Volts, 60 Hz	XX55 000 00	1/pk
Vacuum/Pressure Pump, 110 Volts, 50 Hz	XX55 110 50	1/pk
Vacuum/Pressure Pump, 220 Volts, 50 Hz	XX55 220 50	1/pk
Vacuum Flask, 1 L	XX10 047 05	1/pk
Millex-FG_{50} Filter Unit, 50 mm, 0.2 µm, autoclavable	SLFG 050 10	10/pk
Plate Sealing Tape, opaque	MATA HOP 00	100/pk
Plate Sealing Tape, clear	MATA HCL 00	100/pk
Beckman Manifold Adapter	MAVM MEK 20	

Centrifugal and Chromatography Accessories

	Catalogue Number	Qty/Pack
Column Loader, 25 µL	MACL 096 25	1/pk
Column Loader, 45 µL	MACL 096 45	1/pk
Column Loader, 80 µL	MACL 096 80	1/pk
Column Loader, 100 µL	MACL 096 00	1/pk
Acrylic Scraper for Column Loader, replacement	MACL 0SC 03	3/pk
Centrifuge Alignment Frames, aqueous applications	MACF 096 04	4/pk
Centrifuge Alignment Frames, solvent applications	MACF 096 S4	4/pk

MultiScreen Filtration System Punch Kit, Disposable Punch Tips, and Accessories

	Catalogue Number	Qty/Pack
Multiple Punch, includes punch with (1) punch carrier plate and (1) punch distributor	MAMP 096 08	
Punch Kit A for 7 mL vials, includes (1) Multiple Punch and (1) carrier rack for 7 mL vials	MAPK 896 0A	
Punch Kit B for 4 mL vials, includes (1) Multiple Punch and (1) carrier rack for 4 mL vials	MAPK 896 0B	
Punch Kit C for 12 × 75 mm tubes, includes (1) Multiple Punch and (1) carrier rack for 12 × 75 mm tubes	MAPK 896 0C	
Punch Carrier Plate, replacement	MACP 096 00	1/pk
Punch Distributor, replacement	MAPD 089 60	1/pk
Carrier Racks for 7 mL vials, 12 × 8-place racks with carrying tray	MACR 081 27	1/pk
Carrier Racks for 4 mL vials, 12 × 8-place racks with carrying tray	MACR 081 24	1/pk
Carrier Racks for 12 x 75 mm tubes, 12 × 8-place racks with carrying tray	MACR 812 75	1/pk
Disposable Punch Tips	MADP 196 10	10/pk
Disposable Punch Tips, bulk pack	MADP 196 50	50/pk

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at http://www.millipore.com/techservice.

NOTE: To receive our laboratory products catalogue, call 1-800-MILLIPORE or look us up on the Internet (www.millipore.com). For additional information about MultiScreen plates, including an up-to-date list of applications information and referenced publications, visit us on the World Wide Web at http://www.millipore.com/multiscreen.

Standard Warranty

Millipore Corporation ("Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of Millipore products appearing in Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Millipore's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Millipore promptly of any such breach. If after exercising reasonable efforts, Millipore is unable to repair or replace the product or part, then Millipore shall refund to the customer all monies paid for such applicable product or part. **MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER INDIRECT DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

NOTE: If you use Millipore plates with a vacuum manifold not approved by Millipore, you may void the warranty of the Millipore plates. Call your local Millipore Technical Service office for details.

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