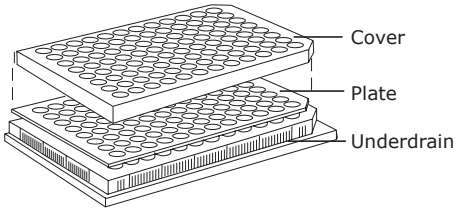
User Guide

MultiScreen® 96-well Plates



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Introduction

The 96-well MultiScreen® plates are designed to filter samples and perform entire procedures, from cell growth to scintillation counting, within the same plate. Use the plates with the MultiScreen® Vacuum Manifold or a similar vacuum manifold. The plates are available in a variety of membrane types, pore sizes, and plate materials. See the "Product Ordering" section for details on available plates.

Use clear plates for general assay applications involving aqueous solutions or low levels of solvents. Use opaque plates for direct micro-scintillation counting and flash luminescence.

MultiScreen® 96-well sterile and non-sterile plates consist of a 96-well plate with integral filters, a plastic underdrain to prevent cross-contamination between wells, and a plastic top cover. Sterile plates come individually blister-packaged to maintain plate sterility.

Wetting Out or Coating Plates Before Use

Some protocols require wetting out or coating of the filter plate prior to use. The "Product Ordering" section lists wetting out requirements. If this is not required for your application, continue on to the "Filtering Samples" section. For ELISpot assays using a plate with Immobilon®-P membrane, wetting out is optional.

Wetting Out Plates

This section describes how to wet out plates with an aqueous or alcohol solution. The solution used will depend on the plate type and assay.

Alcohol Wetting Out for Non-filtration Assays

1. Remove the plate cover.
2. Add 15 µL of 35% ethanol to each well. **Do not vacuum.** Aspirate or "flick" to remove ethanol.
3. Wash twice with 200 µL of starting buffer to flush the residual ethanol from the wells. Remove wash solution as stated above. Do not vacuum. The plate is now ready for sample addition.

NOTE: Once the plate has been wet out, it must be kept damp.

Alcohol Wetting Out for Filtration Assays

1. Place the plate on the manifold and remove the cover.
2. Add 50–100 µL of 70% ethanol to each well. After 30 seconds, filter by applying low vacuum.
3. To flush the residual ethanol from the wells, wash twice with 200 µL of starting buffer, using vacuum. The plate is now ready for sample addition.

NOTE: Once the plate has been wet out, it must be kept damp. Immobilon®-P membrane appears translucent when wet. If the membrane becomes opaque prior to starting the assay, the membrane has dried out and will require rewetting.

Coating

This section describes how to coat the plate with an extracellular matrix (ECM) component.

NOTE: For lymphocytes and suspension cell lines, use plates with Durapore® PVDF membrane. Mixed cellulose ester (HA) plates are recommended for all attachment-dependent cells. HA plates do not usually require coating with a cell adhesion molecule (CAM) or ECM component.

ECM Coating (Sterile Plates)

1. Prepare rat tail collagen (RTC) stock (3 mg/mL) in hydrochloric acid or acetic acid.
2. Dilute 1 part collagen stock with 3 parts 70% sterilized ethanol.
3. Add 40–50 µL aseptically to each well and allow to dry in a laminar flow hood for at least 4 hours or as long as overnight.

NOTE: Dried plates can be sealed and stored dry at 4 °C for up to 4 weeks before running samples.

Sample Addition and Incubation

Seed samples by pipetting the appropriate amount of test sample, from 25 to 250 µL, into each well of the filtration plate. Typical seeding densities are 15,000–40,000 cells/well, depending on the cell line.

When adding multiple solutions to the well, add the solution with the largest volume first, and end by adding the solution containing the smallest volume, if possible. Using this order of addition helps to ensure even mixing of all components.

Cover the filter plate with the plate cover and incubate as required by the application. Do not cover the plate with plate sealing tape because pressure will build up in the wells, causing incubation to fail.

CAUTION: Temperature range for incubation is 4–37 °C.

Filtering Samples

When performing ELISpot applications, the plate does not require filtration and should not be used with the MultiScreen® Vacuum Manifold.

When using FB, and FC plates, the maximum recommended vacuum is 135–271 millibar (4–8 inHg). Always turn the vacuum off between washes to prevent air-locking of plate wells.

For other plates, the maximum recommended vacuum is 271–406 millibar (8–12 inHg). A higher vacuum pressure can be used for difficult-to-filter samples, but this may lead to higher filtrate CV levels and sample foaming.

CAUTION: Do not use the manifold on the same bench or table as a vacuum pump, shaker, or mixer. The vibration may disrupt the filtrate transfer process, impacting quantitative collection of filtrate.

1. Remove the plate cover and add solution(s) to the wells.
2. Replace the plate cover to minimize evaporation. Incubate per assay requirements.
3. Place the plate on the manifold.

   CAUTION: Do not remove the plastic underdrain from the plate before filtering samples. Once the underdrain has been removed, filtrate collection is not possible, even if the underdrain is subsequently replaced.
4. Remove the cover and apply vacuum.

   CAUTION: Empty wells will prevent flow. Add fluid to unused wells or cover unused wells with plate sealing tape.
5. Blot the plate on a lint-free absorbent surface to displace any microdroplets formed on the underside of the plate. Then add any additional solutions that require further incubation.
6. Remove the plastic underdrain for applications that require punching of individual membranes from the plate or for whole-plate scintillation counting situations requiring the addition of a specialized adapter prior to counting.

   CAUTION: To avoid contaminating the samples, do not touch the bottom of the plate.

   See Punching Samples (below) and Whole-plate Scintillation Counting on page 3 for more information.

Punching Samples

Once the assay is complete, samples requiring processing in a counter can be punched using the MultiScreen® Multiple Punch and accessories.

1. Prepare samples per assay requirements. Remove underdrain after the completion of the last step.
2. Dry the plate.

   NOTE: Do not dry HA plates.
3. Load carrier racks with vials or tubes and slide into position on the MultiScreen® Multiple Punch base.
4. Place a MultiScreen® plate (with the underdrain removed) onto the MultiScreen® Plate Carrier Slide.
5. Position the disposable punch tips directly over the 96 wells of the filtration plate. The corner pins and side tabs should fall easily into the positioning grooves on the top of the plate carrier slide.
6. With the punch handle in the upright position, push the plate carrier slide back into the punch through all the detents. Once the plate carrier slide is pushed in as far as it can go, pull it out one detent position on the punch.
7. Push the punch handle down in one rapid motion, causing the disposable punch tips to be driven through each well into the vials or test tubes.
8. Remove the vials, add scintillation fluid if required, and count.

Protocol Notes

- Allowing the glass fiber material to disassociate with shaking prior to counting significantly increases counting efficiency, particularly with tritium labels.
- When using glass fiber plates, care must be taken to regularly disassemble the punch distributor and remove stray glass fibers.
- For glass fiber and HA plates, the supporting membrane may not always be removed with the punch tip, but may instead remain attached to the base plate. The collected counts, however, are contained on the filter.

- Can be used with Packard TopCount® system.

Whole-plate Scintillation Counting

Opaque MultiScreen® plates are compatible with microplate counters for direct plate scintillation counting such as the Packard TopCount® System and Wallac MicroBeta® counter.

1. Perform the assay using opaque MultiScreen® plates according to your typical procedure.
2. Remove the underdrain from the plate and dry the plate to maximize efficiency.
3. Blot plate on lint-free paper towels or other clean absorbent material (optional for faster drying).
4. Place plate in an appropriate holder if necessary.
5. Using a multichannel pipettor, add 25 µL (30 µL for glass fiber plates) of liquid scintillation cocktail to each well.
6. Seal the top of the plate with clear sealing tape.
7. Count.

Chemical Compatibility

Click here for a complete list.

Disposal

Collect and dispose of used material according to all applicable international, federal, state, and local regulations.

Product Ordering

Purchase products online at SigmaAldrich.com.

Plate Description	Pore Size, µm	Wetting Out	Membrane support	Sterile	Qty/pk	Catalogue Number
Plates with hydrophilic Durapore® polyvinylidene fluoride (PVDF) membrane						
MultiScreen®-GV, clear acrylic	0.22	Aqueous solution	N/A	Yes	10	MAGVS2210
MultiScreen®-DV, clear styrene	0.65	Aqueous solution	N/A	No	50	MADVN6550
MultiScreen®-BV, opaque Barex® plastic	1.2	Aqueous solution	N/A	No	50	MABVN0B50
MultiScreen®-BV, clear styrene	1.2	Aqueous solution	N/A	No	50	MABVN1250
Plates with hydrophobic Immobilon® PVDF membrane						
MultiScreen®-IP, clear acrylic	0.45	Ethanol	N/A	No	50	MAIPN4550
MultiScreen®-IP, clear acrylic	0.45	Ethanol	N/A	Yes	10	MAIPS4510
MultiScreen®-IP, white acrylic, no underdrain	0.45	Ethanol	N/A	Yes	10	MAIPSWU10
MultiScreen®-IP, opaque Barex® plastic	0.45	Ethanol	N/A	No	50	MAIPN0B50
Plates with glass fiber filter						
MultiScreen®-FB, opaque Barex® plastic	1.0	Aqueous solution	0.65 µm Durapore® membrane	No	50	MAFBN0B50
MultiScreen®-FC, opaque Barex® plastic	1.2	Aqueous solution	0.65 µm Durapore® membrane	No	50	MAFCN0B50
Plates with mixed cellulose ester (MCE) membrane						
MultiScreen®-HA, clear styrene	0.45	Aqueous solution	N/A	Yes	10	MAHAS4510

Notice

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Technical Assistance

Any serious incident of this device should be reported to manufacturer and competent authority of Country or EU member state where user is established

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

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