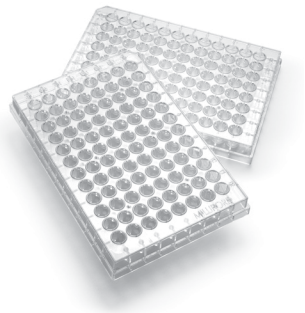
User Guide

MultiScreen®$_{HTS}$ and MultiScreen®$_{HTS}$+ Hi Flow Assay Systems



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Introduction

MultiScreen®$_{HTS}$ 96-well filtration plates are used to filter samples and perform entire procedures, from cell growth to scintillation counting within the same plate. The plates come in many membrane types, pore sizes, and plate materials. The automation-compatible design allows for easy manipulation with a variety of robotics systems and makes barcoding possible on all four sides. Filtration is accomplished either by vacuum using the MultiScreen®$_{HTS}$ Vacuum Manifold (Cat. No. MSVMHTS00), or by centrifugation.

MultiScreen®$_{HTS}$+ Hi Flow 96-well filtration plates are optimal for radiometric kinase and G protein-coupled receptor (GPCR) assays. These plates contain a mesh support in place of a membrane under the active filter. The high flow design of these plates provides the improved flow needed for uniform assay wash steps, reduced background binding, and reduced variation in both signal and background radiometric counts.

Plate Types

Use clear plates for general assay applications involving aqueous solutions or low levels of solvents. Use opaque plates for direct microscintillation counting and flash luminescence. Use solvent resistant plates when working with 30% or greater organic solvent. Sterile plates are sold individually packaged.

Usage Guidelines

- 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.
- Placing sealing tape over all the wells or leaving the cover on the plate while applying vacuum will prevent the flow of liquid through the filters.

Specifications

Filter plate well capacity	300 µL
Working sample volume (capacity may be limited by receiver plate)	250 µL
Active Membrane Area	0.2 cm^2 (0.03 in^2)
Centrifugal speed (maximum)	3000 × g
Dimensions, filter plate	
Length	127.8 mm (5.0 in.)
Width	85.5 mm (3.4 in.)
Depth	14.4 mm (0.6 in.)
Membrane	GV 0.22 µm hydrophilic Durapore® PVDF membrane
	HV 0.45 µm hydrophilic Durapore® PVDF membrane
	DV 0.65 µm hydrophilic Durapore® PVDF membrane
	BV 1.2 µm hydrophilic Durapore® PVDF membrane
	IP 0.45 µm hydrophobic Immobilon®-P PVDF membrane
	HA 0.45 µm hydrophilic mixed cellulose ester membrane
	PCF 0.4 µm polycarbonate membrane
	FB 1.0 µm glass fiber filter
	FC 1.2 µm glass fiber filter

Wetting Out or Coating Plates Before Use

Some protocols require wetting out or coating of the filter plate prior to use. If this is not required for your application, continue on to the "Filtering Samples" section.

Wetting Out Plates

Wet out plates with an aqueous or alcohol solutions. 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 well. 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. 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.

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.

Alcohol Wetting Out for Cell Staining

1. Place the plate on the manifold and remove the cover.
2. According to stain/destain manufacturer protocol, apply stain to each well and incubate. Remove stain by applying low vacuum. Repeat if wash step needed.
3. If filters are to be used for analysis or manually extracted, underdrains can be removed in step 4.
4. Carefully remove underdrain using curved nose type of needle nose plier using rolling motions.

Colony Counting Assays When De-Staining, and Underdrain Removal

1. Place the plate on manifold and remove the cover.
2. According to stain/de-stain manufacturer's protocol, apply stain to each well and incubate. Remove stain by applying low vacuum.
3. Plates can be de-stained using commercially available de-stains.

   CAUTION: De-stain should not contain more than 50% alcohol.
4. Remove de-stain by applying low vacuum (< 10 inHg).
5. Carefully remove underdrain using curved nose type of needle nose plier using rolling motions.

Coating Plates

Coat the plate with an extracellular matrix (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®$_{HTS}$ Vacuum Manifold.

When using MultiScreen®$_{HTS}$+ Hi Flow-FB, -FC plates, the maximum recommended vacuum is 135–271 millibar (4–8 inHg).

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.

When using glass fiber plates, always turn the vacuum off between washes to prevent air-locking of the plate wells.

CAUTION: Do not use the manifold on the same bench or table with 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. Whole plate scintillation counting can be performed with underdrains attached or removed. Some counting situations require the addition of a specialized adapter for counting equipment.

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

See "Whole Plate Scintillation Counting" section for more information.

Whole Plate Scintillation Counting

Opaque MultiScreen®$_{HTS}$ plates are compatible with microplate counters for direct plate scintillation counting.

1. Perform the assay using opaque MultiScreen®$_{HTS}$ 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.

Protocol Notes

- Allowing the glass fiber material to disassociate with shaking prior to counting significantly increases counting efficiency, particularly with tritium labels.
- MultiScreen®$_{HTS}$+ Hi Flow filter plates are not compatible with the MultiScreen® Multiple Punch.

Chemical Compatibility

The chemical compatibility list has been moved online for your convenience. Visit SigmaAldrich.com/compatibility.

Product Ordering

Purchase products online at SigmaAldrich.com/products.

Plates with Hydrophilic Durapore® Polyvinylidene Fluoride (PVDF) Membrane

Plate Description	Pore Size, µm	Sterile	Qty/Pk	Catalogue Number
MultiScreen®$_{HTS}$-GV, clear styrene	0.22	No	10 50	MSGVN2210 MSGVN2250
MultiScreen®$_{HTS}$-GV, clear styrene	0.22	Yes	10	MSGVS2210
MultiScreen®$_{HTS}$-GV, opaque Barex® plastic	0.22	No	50	MSGVN2B50
MultiScreen®$_{HTS}$-HV, clear styrene	0.45	No	10 50	MSHVN4510* MSHVN4550*
MultiScreen®$_{HTS}$-HV, clear styrene	0.45	Yes	10	MSHVS4510
MultiScreen®$_{HTS}$-HV, opaque Barex® plastic	0.45	No	10 50	MSHVN4B10 MSHVN4B50
MultiScreen®$_{HTS}$-DV, clear styrene	0.65	No	10 50	MSDVN6510 MSDVN6550
MultiScreen®$_{HTS}$-DV, opaque Barex® plastic	0.65	No	50	MSDVN6B50
MultiScreen®$_{HTS}$-BV, clear styrene	1.2	No	10 50	MSBVN1210 MSBVN1250
MultiScreen®$_{HTS}$-BV, clear styrene	1.2	Yes	10	MSBVS1210
MultiScreen®$_{HTS}$-BV, opaque Barex® plastic	1.2	No	50	MSBVN1B50

* For in vitro diagnostic use

Plates with Hydrophobic Immobilon®-P PVDF Membrane

Plate Description	Pore Size, µm	Sterile	Qty/Pk	Catlogue Number
MultiScreen®$_{HTS}$-IP, clear acrylic	0.45	No	10 50	MSIPN4510 MSIPN4550
MultiScreen®$_{HTS}$-IP, clear acrylic	0.45	Yes	10	MSIPS4510
MultiScreen®$_{HTS}$-IP, white acrylic	0.45	Yes	10	MSIPS4W10
MultiScreen®$_{HTS}$-IP, opaque Barex® plastic	0.45	No	10 50	MSIPN4B10 MSIPN4B50
MultiScreen® 8-well Strip with Immobilon®-P membrane	0.45	Yes	10 × 96-well plates	M8IPS4510
MultiScreen® 8-well Strip Support Frame	N/A	N/A	10	M8IPFRAME

Plates with Hydrophilic Mixed Cellulose Esters (MCE) Membrane

Plate Description	Pore Size, µm	Sterile	Qty/Pk	Catalogue Number
MultiScreen®$_{HTS}$-HA, clear styrene	0.45	No	10 50	MSHAN4510 MSHAN4550
MultiScreen®$_{HTS}$-HA, clear styrene	0.45	Yes	10	MSHAS4510
MultiScreen®$_{HTS}$-HA, opaque Barex® plastic	0.45	No	50	MSHAN4B50
MultiScreen®$_{HTS}$-HA, opaque Barex® plastic	0.45	Yes	10	MSHAS4B10

Specialty Membranes and Filters

Plate Description	Pore Size, µm	Membrane support	Sterile	Qty/Pk	Catalogue Number
Plates with polycarbonate membrane, for aqueous, small molecule filtration and sample prep MultiScreen®$_{HTS}$-PCF, clear styrene	0.4	N/A	No	10 50	MSSLBPC10 MSSLBPC50
Plates with glass fiber filter MultiScreen®$_{HTS}$+ Hi Flow-FB, opaque Barex® plastic	1.0	Polyester mesh	No	50	MSFBNXB50
MultiScreen®$_{HTS}$+ Hi Flow-FC, opaque Barex® plastic	1.2	Polyester mesh	No	50	MSFCNXB50
MultiScreen®$_{HTS}$-FB, opaque Barex® plastic	1.0	0.65 µm Durapore® membrane	No	10 50	MSFBN6B10 MSFBN6B50
MultiScreen®$_{HTS}$-FC, opaque Barex® plastic	1.2	0.65 µm Durapore® membrane	No	10 50	MSFCN6B10 MSFCN6B50

Accessories

Description	Qty/Pk	Catalogue Number
MultiScreen®$_{HTS}$ Vacuum Manifold Includes manifold base, standard collar, gaskets, gasket inserts, tubing, valves, and pressure gauge	1	MSVMHTS00
Vacuum Manifold Kits Manifold Kits include MultiScreen®$_{HTS}$ Vacuum Manifold, chemical duty pump (choose appropriate voltage), vacuum flask, stoppers, and Millex® filters Vacuum manifold kit (220 volts, 50 Hz)	1	MSVMKIT00
Vacuum manifold kit (115 volts, 60 Hz)	1	MSVMKIT01
Vacuum manifold kit (100 volts, 50/60 Hz)	1	MSVMKIT02
Vacuum Manifold Kit Components		
MultiScreen®$_{HTS}$ Vacuum Manifold	1	MSVMHTS00
Chemical duty pump (220 volts, 50 Hz)	1	WP6122050
Chemical duty pump (115 volts, 60 Hz)	1	WP6111560
Chemical duty pump (100 volts, 50/60 Hz)	1	WP6110060
Vacuum flask, 1 L	1	XX1014705
#8 Silicone stoppers, 9.5 mm hole	5	XX2014718
Millex®-FA$_{50}$ filter unit	10	SLFA05010

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