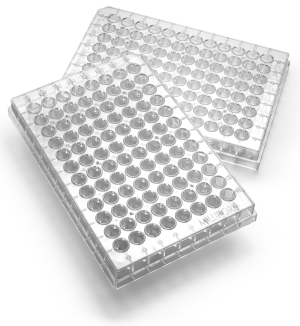
User Guide

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



For filtering samples and performing entire procedures, from cell growth to scintillation counting in the same plate

For research use only.
Not for use in diagnostic procedures.

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 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.

Specifications for 96-well Plate Format

Filter plate well capacity	300 µL
Working sample volume (capacity may be limited by receiver plate)	250 µL
Centrifugal speed (maximum)	3000 × g
Dimensions, filter plate	
Length	123.4 mm (4.9 in.)
Width	82.7 mm (3.3 in.)
Depth	14.6 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
	PH negatively charged phosphocellulose membrane
	DE positively charged DEAE membrane
	FB 1.0 µm glass fiber filter
	FC 1.2 µm glass fiber filter

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.

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

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.

Aqueous Wetting Out for PH and DE Plates

1. Place the plate on the manifold and remove the cover.
2. Add 100 µL of starting buffer to each well.
3. Apply vacuum after 1 minute. The plate is now ready for sample addition.

NOTE: Once PH and DE plates have been wet out, they must be used immediately. The filters will shrink upon drying and may fall out of the wells.

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. For more information, see publication MM012 at www.millipore.com (*enter MM012 in the search box*).

ECM Coating (Sterile Plates)

1. Prepare rat tail collagen (RTC) stock (3 mg/mL) in hydrochloric acid or acetic acid. For more information on coating with other ECM components, see publication MM012.
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 four 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. For detailed protocols, see publication TN1003EN00 at www.millipore.com (*enter TN1003EN00 in the search box).*

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

For other plates, the maximum recommended vacuum is 271–406 millibar (8–12 in. Hg). 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, PH, or DE 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. 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 adaptor prior to counting.

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

   See "Punching Samples" and "Whole Plate Scintillation Counting" sections 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. The MultiScreen®$_{HTS}$ Plate Carrier Slide (cat. no. MSCP09600) is required for punching samples from MultiScreen®$_{HTS}$ plates. For more information, refer to the MultiScreen® Separations System User Guide, P17479, available at www.millipore.com (*enter P17479 in the search box*). For guidelines on low-energy isotope detection, consult publication MM010 at www.millipore.com (*enter MM010 in the search box*).

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

   NOTE: Do not dry HA, PH, and DE plates.
3. Load carrier racks with vials or tubes and slide into position on the MultiScreen® Multiple Punch base.
4. Place a MultiScreen®$_{HTS}$ plate (with the underdrain removed) onto the MultiScreen®$_{HTS}$ Plate Carrier Slide.

Punching Samples, continued

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, PH, and DE 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.
- MultiScreen®$_{HTS}$+ Hi Flow filter plates are not compatible with the MultiScreen® Multiple Punch.

Whole Plate Scintillation Counting

Opaque MultiScreen®$_{HTS}$ plates are compatible with microplate counters for direct plate scintillation counting. For more detailed protocols see MM015 for the Wallac MicroBeta® counter and TN020 for the Packard TopCount® system. These publications can be found at www.millipore.com (*enter the publication number in the search box*).

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. For counting with the underdrain in place, refer to publication TN020.
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, PH, and DE plates) of liquid scintillation cocktail to each well.
6. Seal the top of the plate with clear sealing tape.
7. Count.

Chemical Compatibility

Chemical compatibility for MultiScreen®$_{HTS}$ plates and accessories can be found in publication PR4772EN00 or PR4772ENEU, available at www.millipore.com (*enter the publication number in the search box*).

Ordering Information

Plates with hydrophilic Durapore® polyvinylidene fluoride (PVDF) membrane

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

* For in vitro diagnostic use

Plates with hydrophobic Immobilon®-P PVDF membrane

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

Plates with hydrophilic mixed cellulose esters (MCE) membrane

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

Ordering Information, continued

Specialty Membranes and Filters

	Plate Description	Pore Size, µm	Membrane support	Sterile	Cat. No.	Qty/Pk
Specialty Membranes and Filters	Plates with polycarbonate membrane, for aqueous, small molecule filtration and sample prep					
	MultiScreen®$_{HTS}$-PCF, clear styrene	0.4	N/A	No	MSSLBPC10 MSSLBPC50	10 50
	Plates with negatively charged (phosphocellulose) membrane					
	MultiScreen®$_{HTS}$+ Hi Flow-PH, opaque Barex® plastic	- charge	Polyester mesh	No	MSPHNXB50	50
	MultiScreen®$_{HTS}$-PH, opaque Barex® plastic	- charge	0.65 µm Durapore® membrane	No	MSPHN6B10 MSPHN6B50	10 50
	Plates with positively charged DEAE membrane					
	MultiScreen®$_{HTS}$-DE, opaque Barex® plastic	+ charge	0.65 µm Durapore® membrane	No	MSDEN6B50	50
	Plates with glass fiber filter					
	MultiScreen®$_{HTS}$+ Hi Flow-FB, opaque Barex® plastic	1.0	Polyester mesh	No	MSFBNXB50	50
	MultiScreen®$_{HTS}$+ Hi Flow-FC opaque Barex® plastic	1.2	Polyester mesh	No	MSFCNXB50	50
	MultiScreen®$_{HTS}$-FB, opaque Barex® plastic	1.0	0.65 µm Durapore® membrane	No	MSFBN6B10 MSFBN6B50	10 50
	MultiScreen®$_{HTS}$-FC, opaque Barex® plastic	1.2	0.65 µm Durapore® membrane	No	MSFCN6B10 MSFCN6B50	10 50

Accessories

	Description	Cat. No.	Qty/Pk
Accessories	MultiScreen®$_{HTS}$ Vacuum Manifold		
	Includes manifold base, standard collar, gaskets, gasket inserts, tubing, valves, and pressure gauge	MSVMHTS00	1
	Vacuum Manifold Kits		
	Manifold kits include MultiScreen®$_{HTS}$ Vacuum Manifold, chemical duty pump (choose appropriate voltage), vacuum flask, stoppers, and Millex® filter units		
	Vacuum manifold kit (220 volts, 50 Hz)	MSVMKIT00	1
	Vacuum manifold kit (115 volts, 60 Hz)	MSVMKIT01	1
	Vacuum manifold kit (100 volts, 50/60 Hz)	MSVMKIT02	1
	Vacuum Manifold Kit Components		
	MultiScreen®$_{HTS}$ Vacuum Manifold	MSVMHTS00	1
	Chemical duty pump (220 volts, 50 Hz)	WP6122050	1
	Chemical duty pump (115 volts, 60 Hz)	WP6111560	1
	Chemical duty pump (100 volts, 50/60 Hz)	WP6110060	1
	Vacuum flask, 1 L	XX1004705	1
	#8 Silicone stoppers, 9.5 mm hole	XX2004718	5
	Millex®-FA$_{50}$ filter unit	SLFA05010	10
	MultiScreen® Punch Kit and Accessories		
	MultiScreen® Multiple Punch	MAMP09608	1
	Plate Carrier Slide for MultiScreen®$_{HTS}$ Filter Plates	MSCP09600	1
	MultiScreen® Disposable Punch Tips (array of 96 tips)	MADP19650	50

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