

- ▶ Sensitive, specific and reliable methodology
- ▶ Fast assay set up
- ▶ Easy scale-up from 96- to 384-well assays
- ▶ Plates compatible with ANSI/SBS 2004 standards for microwell plates

MultiScreen[®]_{HTS}-PH Phosphocellulose Filter Plates

384- and 96-well plates incorporate optimized phosphocellulose paper for high throughput radiometric kinase assays

Most Reliable Tool for Protein Kinase Screening Assays

Filter-based separation technology is widely used to obtain highly reproducible, reliable and quantitative results in kinase screening assays. This is a critical step in lead identification and later lead characterization processes.

By providing a direct measure of phosphorylation, radiometric assays are a proven and direct method for kinase screening. They are highly sensitive, specific and reliable, and provide a superior alternative to homogenous assays. In addition, phosphocellulose paper is widely recognized for having high peptide binding properties and very low non-specific binding.

Because radiometric methodology is not subject to naturally fluorescent or quenching compounds, it is not susceptible to false positives or negative results.

Fast Assay Set-up

MultiScreen_{HTS}-PH filter plates are easy to set up for kinase assays. The optimized platform and protocol eliminates the need to design, develop, and optimize specific combinations of reagents. Protocols and application

notes for this application are available from Millipore (for more information, request the following: PC0027EN00: Protein Kinase Assays on MultiScreen_{HTS}-PH 384-well Filter Plates, AN1017EN00: Kinase Assays Performed Entirely in the MultiScreen_{HTS}-PH 384-well Filter Plate, or AN1018EN00: Use of MultiScreen_{HTS}-PH 384-well Filter Plates on the Wallac MicroBeta[®] Trilux Plate Reader to Achieve Optimal Radiometric Assay Results).

Innovative Plate Design

MultiScreen_{HTS} filter plates are fully compatible with automation and meet the 2004 ANSI/SBS standards for multiwell plates. For high throughput kinase screening assays, MultiScreen_{HTS}-PH plates incorporate phosphocellulose paper to provide an optimized platform for reliable in-plate radiometric analysis.

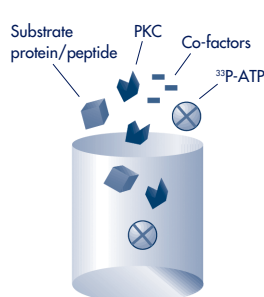
The new HTS design for both 96- and 384-well plates features rigid sidewalls aligned for robotic gripper arms. They also provide ample surfaces for bar code labels. The plate design eliminates surface contact with individual well "drip directors." A plastic skirt improves the vacuum seal when used with a Millipore_{HTS} vacuum manifold (Millipore Cat. No. MSVM HTS 00).

Easy Scale-up from 96-well Platforms

MultiScreen_{HTS}-PH is available in both 96- and 384-well formats for maximum versatility. The 384-well filter plate offers the reagent-saving benefits of assay miniaturization and is a simple scale-up from 96-well assays.

Details on scale-up are provided in Millipore's Kinase Screening Assay Application Note (AN2040EN00) and Protocol Note (PC2040EN00). Both are available online at www.millipore.com/HTS.

Protein Kinase A Assay



1. Prewet with 100 μ L of 1M Tris. Add protein kinase A (PKA), 33 P-ATP, substrate peptide and co-factors to MultiScreen_{HTS} filter plate. Incubate to allow PKA to transfer 33 P from ATP to substrate peptide.



2. Add phosphoric acid to solution.



3. Collect all peptide on filter. Wash through free ATP, etc. Add scintillant and seal with tape.

Optimized Design for High Quality Results

Counting Efficiency and Signal-to-Noise

Plate Type	Plate Reader Mode	% Efficiency at 100K DPM	33 P Ratio Signal/Noise
384-well MZPH	Coincidence Counting	35.6	2937:1
384-well MZPH	Top PMT only	49.5	270:1
384-well MZPH	Bottom PMT only	19.3	266:1
96-well MSPH	Coincidence PMT only	62.4	2263:1
96-well MSPH	Top PMT only	69.0	248:1
96-well MSPH	Bottom PMT only	54.5	12:1

Conditions: PE Trilux with 33 P γ -ATP with 10 μ L Supermix

Table 1. Radiometric counting efficiencies and signal-to-background values for approximately 100,000 DPM 33 P labeled compounds as determined in the Wallac MicroBeta Trilux Counting Instrument with 10 μ L Wallac Optiphase Supermix scintillation cocktail in 96- and 384-well PH filter plates. 96-well filter plates were counted with the under-drain on. **NOTE:** MultiScreen_{HTS}-PH filter plates should not be dried prior to LSC addition.

Peptide Binding Capacity

Plate	Number of Wells	Peptide Binding Capacity ¹	Protein Binding Capacity ²
MSPH	96-well	300 ng	18 µg
MZPH	384-well	110 ng	6 µg

Table 2. Typical peptide and protein binding capacities were determined in 96- and 384-well MultiScreen_{HTS} phosphocellulose filter plates. Washing was performed with sodium acetate buffer (50 mM pH 4.5).

- ¹ Peptide binding was determined with tritium labeled neurotensin by counting retained radioactivity after washing.
- ² Protein binding was determined with bovine serum albumin (BSA) by colorimetric analysis of protein after elution with sodium chloride (0.5M pH 7.4) buffer.

Protein Kinase A Activity

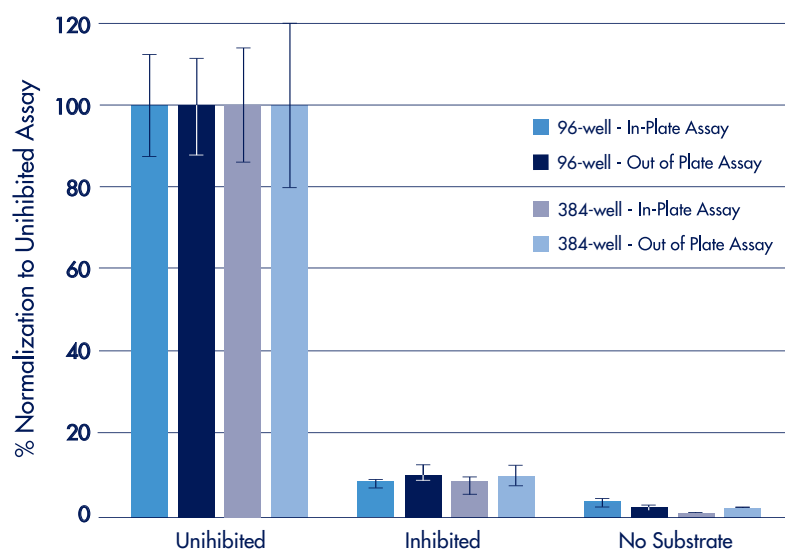


Figure 1. The MultiScreen_{HTS} filter plate demonstrates consistent results in both 96- and 384-well assays for in-plate and out-of-plate assays. The in-plate assay eliminates the need for reaction transfer and allows for reactions to be initiated, incubated, terminated, washed and counted in a single MultiScreen filter plate. This reduces radioactive waste and streamlines the assay. For more information on in-plate 384-well assays, refer to Millipore Application Note AN1017EN00.

Specifications

	MultiScreen _{HTS} 384	MultiScreen _{HTS} 96
Materials of Construction		
Filter material:	Phosphocellulose paper	Phosphocellulose paper
Support:	Polyester Mesh	0.65 Durapore® PVDF
Filter plate:	White styrene acrylonitrile (San)/TiO ₂	White Barex/TiO ₂
Flow director:	Clear SAN	Natural polyethylene
Plate lid:	Polystyrene	Polystyrene
Dimensions		
Plate assembly length:	127.7	127.7
Plate assembly width:	85.5	85.5
Plate assembly depth (without lid):	14.4	14.4
Plate assembly depth (with lid):	16.9	16.9
Filter surface area:	0.09 cm ²	0.26 cm ²
Sample Volume per Well		
Recommended:	20 – 100 µL	50 – 250 µL
Maximum:	110 µL	300 µL
Hold-up after Vacuum Filtration		
	3 µL	6 µL
Filtration Parameters		
Recommended vacuum pressure:		
Filter to waste:	4 – 12" Hg	4 – 12" Hg
Filter to receiver plate:	4 – 8" Hg	4 – 8" Hg
Recommended centrifugal force:	500 – 1000 x g	1000 x g

Ordering Information

Description	Qty/Pk.	Catalogue No.
MultiScreen _{HTS} 384-well filter plates with Phosphocellulose	10	MZPH NOW 10
	50	MZPH NOW 50
MultiScreen _{HTS} PH 96-well filter plates with Phosphocellulose	10	MSPH N6B 10
	50	MSPH N6B 50

Accessories

MultiScreen _{HTS} Vacuum Manifold, 1 unit		MSVM HTS 00
Chemical duty vacuum pump,	115 Volts, 60 Hz	WP61 115 60
	220 Volts, 50 Hz	WP61 220 50
Plate sealing tape	Clear	MATA HCL 00
	Opaque	MATA HOP 00
Vacuum flask, 1 L		XX10 047 05

Related Information

PF1544EN00: MultiScreen_{HTS} Filter Plates Data Sheet

AN1017EN00: Kinase Assays Performed Entirely in the MultiScreen_{HTS}-PH 384-well Filter Plate Application Note

AN1018EN00: Use of MultiScreen_{HTS}-PH 384-well Filter Plates on the Wallac MicroBeta Trilux Plate Reader to Achieve Optimal Radiometric Assay Results Application Note

AN457: Second Messenger Assays Using the MultiScreen System Application Note

MM021: The MultiScreen Guide to Filtration Based Enzyme Assays

MM014: Guidelines for BioAssays on MultiScreen Filter Plates

PC0027EN00: Protein Kinase Assays on 384-well MultiScreen_{HTS}-PH Filter Plates

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