Novel radioligand binding assays with GPCR membranes prefrozen on MultiScreen®$_{HTS}$ glass fiber filter plates (Ready-To-Assay™ plates)

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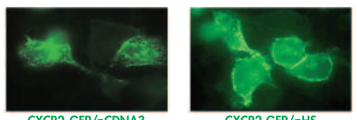
Abstract

The use of glass fiber filter plates remains a popular platform for performing radioligand binding assays on GPCR membrane preparations because signal:background is typically higher than with other platforms. However, the multiple steps involved in performing filtration radioligand binding assays results in lower throughput. We have developed a method for preparing MultiScreen®$_{HTS}$ glass fiber filter plates that are supplied preloaded with GPCR membrane preparation. Because the filter plate has sufficient volume and low nonspecific ligand binding, the end user is able to add just compound and radioligand to initiate the binding reaction within the filter plate. We have validated this platform with a variety of receptors from different classes, including receptors for adenosine (A3), prostanoids (EP3 and TP), and peptides (NMU1 and V1a). Radioligand binding assays with these GPCR membranes prefrozen on MultiScreen plates have a signal:background comparable to assays performed in a separate reaction plate and transferred to a harvest plate. In addition, the Bmax of the membranes prefrozen on the filter plates is comparable to Bmax values obtained with a reaction performed in a separate plate. The membranes remain highly stable during storage within the filter plates. As a result, the GPCR membrane preparations stored prefrozen in MultiScreen®$_{HTS}$ filter plates provide a significant savings of time with no sacrifice in quality of the resulting data. In addition, this platform offers the potential to create GPCR membrane arrays, in which multiple targets can be provided on one plate to allow the user to easily profile compounds against a family of GPCRs.

Millipore's ChemiScreen™ GPCR technology

Fluorescent imaging:



CXCR2-GFP/pCDNA3 transfected in CHO (Conventional)

CXCR2-GFP/pHS transfected in Chem-1 (Chemicon)

1. Novel mammalian expression system to express more GPCR on cell surface
2. High cell surface expression results in excellent pharmacology and signal:background

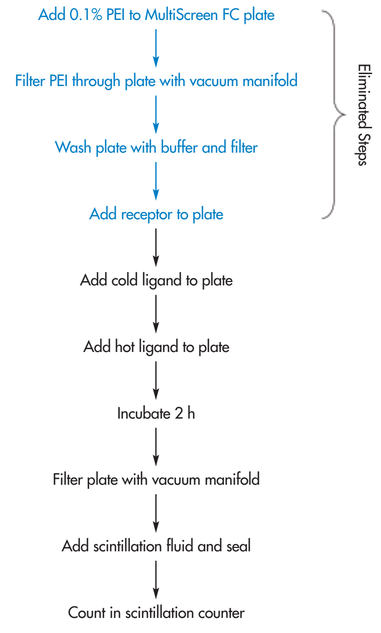
Millipores's MultiScreen®$_{HTS}$ Filter Plate technology

1. The deep wells of the MultiScreen$_{HTS}$ filter plates permit binding reactions to be performed in the filter plate
2. Vacuum manifold is used for washing the plates

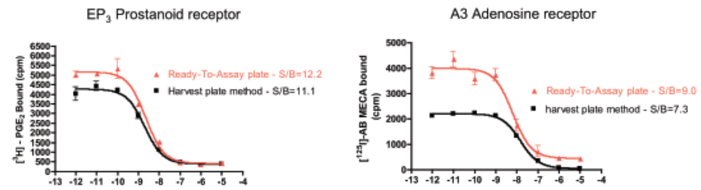
Ready-To-Assay™ Plates

- Employs ChemiScreen™ GPCR Membrane Preparations
- Novel technology to preload and preserve GPCR membrane preparations prefrozen in PEI-coated MultiScreen$_{HTS}$ FC plates
- Plated at an optimized membrane concentration
- Simple to use – just thaw plate then add compounds and radioligand
- Available with single receptor per plate for screening or extensive pharmacology
- Also available with a family of receptors on one plate, such as the Prostanoid Receptor Array
- Validated for high signal/background and optimal pharmacology in radioligand binding assay

Ready-To-Assay™ plates eliminate four steps from conventional filtration binding assay:



Comparable S/B in GPCR Radioligand Binding with Ready-To-Assay™ plates and conventional harvest plate method

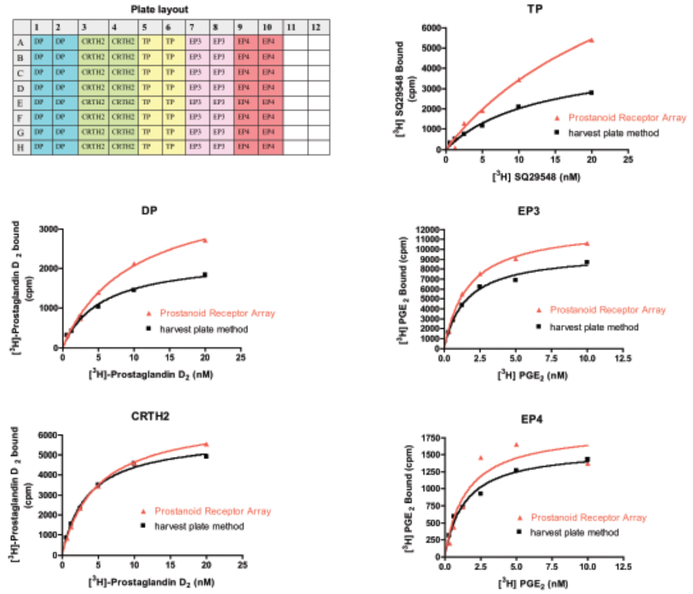


Prostanoid Receptor Array for Convenient Profiling Against a Family of GPCRs

Specific binding of radiolabeled ligands to prostanoid receptor membrane preparations prefrozen in a single MultiScreen$_{HTS}$ FC glass fiber filter plate

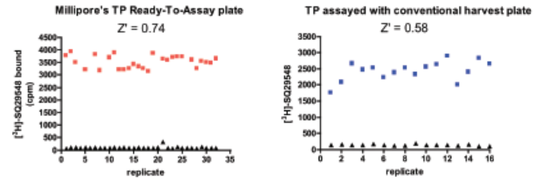
Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
B	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
C	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
D	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
E	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
F	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
G	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		
H	DP	DP	CRTH2	CRTH2	TP	TP	EP3	EP3	EP4	EP4		



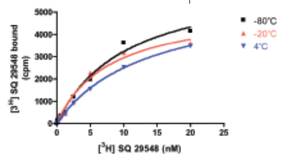
Z′ values are preserved with Ready-To-Assay™ Plates

Radioligand binding assay for TP Prostanoid Receptor



Stability of Ready-To-Assay™ Plates

Specific binding of labeled PGE_2 to EP3 membranes loaded onto MultiScreen$_{HTS}$ FC plates and stored at indicated temperature for 15 days



Summary

Advantages of Ready-to-Assay™ Membranes on MultiScreen®$_{HTS}$ Filter Plates

- In-plate reaction omits need for a separate incubation plate
- No need to precoat filter plate with PEI or to dilute, optimize and plate GPCR membranes
- GPCR Receptor Arrays provide a convenient method to analyze compounds against a GPCR family
- Pharmacology and signal are comparable to conventional methods

Products Available from Millipore

HTS900PA Prostanoid Receptor Array
HTS092P EP3 Ready-To-Assay™ plate, 96-well
HTS091P DP Ready-To-Assay™ plate, 96-well
HTS031P CRTH2 Ready-To-Assay™ plate, 96-well
HTS081P TP Ready-To-Assay™ plate, 96-well
MultiScreen$_{HTS}$ Plus 96-well glass fiber filter (catalog #MSFC X6B)
ChemiScreen™ GPCR Membrane Preparations (catalog #HTS001M-HTS185M) will soon be available in Ready-To-Assay™ plate format, with a single receptor or multiple receptors per plate

References

Birzin ET and Rohrer SP (2002) High-throughput receptor-binding methods for somatostatin receptor 2. Anal. Biochem. 307: 159-166

Harms A et al. (2000) Development of a 5-hydroxytryptamine2A receptor binding assay for high throughput screening using 96-well microfilter plates. J. Biomol. Screen. 5: 269-277.

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