

Abstract

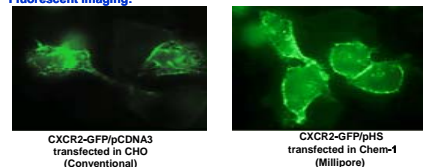
Filtration based radioligand binding assays with receptor-expressing membrane preparations typically obtain higher signal-to-background ratios than other platforms, but throughput is lower with filtration because of the extra steps required. Millipore has recently developed and released the Ready-To-Assay[™] Pre-Plated Membrane Preps platform consisting of GPCR membrane preparations pre-frozen in MultiScreen_{HTS} glass fiber filter plates. With the increased capacity and low nonspecific ligand binding of the MultiScreen_{HTS} plates, the end user only needs to add compound of interest and radioligand to begin the binding reaction. After reaction reaches equilibrium, filtration is performed in the same assay plate to avoid additional transfer steps. We have extended this platform to include Ready-To-Assay Preps in plates containing a series of alpha and beta adrenergic receptor-expressing membrane preparations. We demonstrate that the signal:background ratios, Z' values, Bmax and pharmacology of the adrenergic receptors assayed in the Ready-To-Assay Preps in plate format are comparable to those obtained with conventional filtration binding assays performed in an assay plate and transferred to a separate harvest filter plate. As a result, the adrenergic receptor Ready-To-Assay Preps in plates enable improved workflow without affecting the signal or pharmacology of the adrenergic receptors.

Introduction

Millipore's ChemiScreen[™] GPCR technology

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

Fluorescent imaging:



Millipore's MultiScreen_{HTS} Filter Plate technology

1. The wells of the MultiScreen_{HTS} + Hi Flow filter plates permit binding reactions to be performed in the filter plate
2. Vacuum manifold is used for washing the plates
3. Both filter plates and vacuum manifold are designed to be used with standard automation equipment

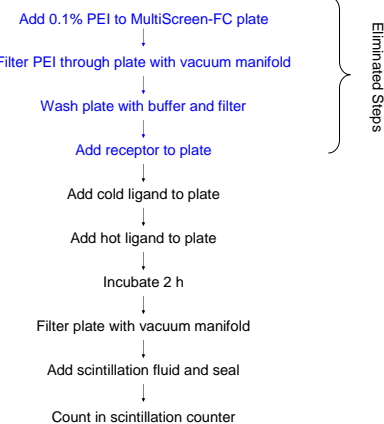


Assay Overview

Millipore's Ready-to-Assay Pre-Plated Membrane Preps

- Employs ChemiScreen GPCR Membrane Preparations pre-plated at an optimized membrane concentration
- Novel technology to pre-load and preserve GPCR membrane preparations pre-frozen in PEI-coated MultiScreen_{HTS}-FC plates
- Simple to use – just thaw plate then add compounds and radioligand
- Available with single receptor per plate for screening or family of receptors on a single plate for selectivity profiling
- Validated for high signal/background and optimal pharmacology in radioligand binding assay

Ready-To-Assay preps eliminate four steps from conventional filtration binding assays:



Methods

Ready-to-Assay Prep method:

The plates were thawed in a 37°C incubator. Radiolabeled ligands and test compounds were added to the plate at the concentrations indicated. The reaction was incubated for 2 h at room temperature. The plates were filtered using a MultiScreen_{HTS} Vacuum Manifold and washed 3 times with Wash Buffer, 300 µL/well/wash. The underdrain was removed and the plates were dried. Scintillation cocktail was added, and the plates were counted in coincidence mode

Harvest Plate method: Membrane preparations were thawed rapidly and chilled on ice. A binding reaction consisting of unlabeled compounds at the concentrations indicated, radioligand, and 5 µg/well membrane preparation in binding buffer was assembled in an assay plate (Corning). The reaction was incubated for 2 h at room temperature, during which time a MultiScreen Harvest Plate (Millipore cat. # MAHF C1H 60) was incubated for 15 min with 0.3% PEI and washed with 50mM HEPES, pH 7.4, 500mM NaCl. Binding reaction was transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate was dried and scintillation cocktail added. The plate was counted on top read mode.

Results

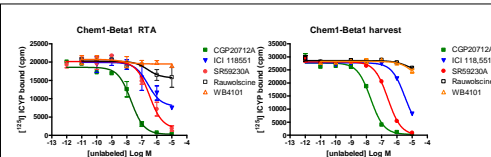


Figure 1. Comparison of rank ordering of adrenoceptor ligands with β_1 membranes in Ready-to-Assay and conventional formats.

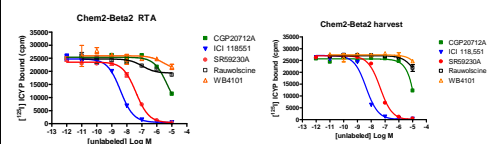


Figure 2. Comparison of rank ordering of adrenoceptor ligands with β_2 membranes in Ready-to-Assay and conventional formats.

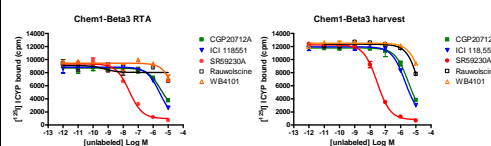


Figure 3. Comparison of rank ordering of adrenoceptor ligands with β_3 membranes in Ready-to-Assay and conventional formats.

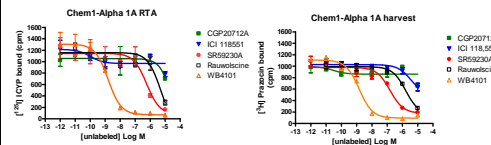


Figure 4. Comparison of rank ordering of adrenoceptor ligands with α_{1A} membranes in Ready-to-Assay and conventional formats.

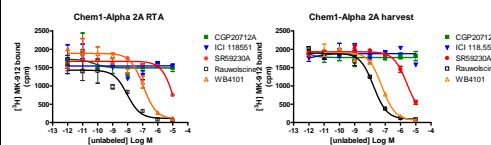


Figure 5. Comparison of rank ordering of adrenoceptor ligands with α_{2A} membranes in Ready-to-Assay and conventional formats.

Conclusion

Rank order of compounds at each adrenoceptor was as follows:

β_1 Ready-to-Assay: CGP20712A>SR59230A>ICI118551>Rauwolfscine>WB4101
 β_1 harvest method: CGP20712A>SR59230A>ICI118551>Rauwolfscine>WB4101

β_2 Ready-to-Assay: ICI118551>SR59230A>>CGP20712A>Rauwolfscine>WB4101
 β_2 harvest method: ICI118551>SR59230A>>CGP20712A>Rauwolfscine>WB4101

β_3 Ready-to-Assay: SR59230A>>ICI118551=CGP20712A>Rauwolfscine=WB4101
 β_3 harvest method: SR59230A>>ICI118551=CGP20712A>Rauwolfscine=WB4101

α_{1A} Ready-to-Assay: WB4101>>SR59230A>Rauwolfscine>CGP20712A=ICI118551
 α_{1A} harvest method: WB4101>>SR59230A>Rauwolfscine>CGP20712A=ICI118551

α_{2A} Ready-to-Assay: Rauwolfscine>WB4101>SR59230A>>CGP20712A=ICI118551
 α_{2A} harvest method: Rauwolfscine>WB4101>SR59230A>>CGP20712A=ICI118551

With each adrenoceptor, rank order was preserved between Ready-to-Assay plate and harvest plate methods

Related Products

- HTS104P β_1 Ready-to-Assay Plate, 96-well
- HTS073P β_2 Ready-to-Assay Plate, 96-well
- HTS159P β_3 Ready-to-Assay Plate, 96-well
- HTS087P α_1A Ready-to-Assay Plate, 96-well
- 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
- HTS001M – HTS208M, over >110 different GPCR Membrane preps available

Filter plates and accessories:

- MultiScreen_{HTS} + Hi Flow 96-well glass fiber filter (catalog #MSFC NX B50)
- MultiScreen_{HTS} Vacuum Manifold (catalog # MSVM HTS 00)

Summary

Advantages of Millipore's Ready-to-Assay Pre-Plated Membrane Preps

- o Pharmacology and signal for adrenergic receptor Ready-to-Assay Preps are comparable to conventional methods
- o In-plate reaction omits the need for a separate incubation plate
- o No need to pre-coat filter plate with PEI or to dilute, optimize and plate GPCR membranes
- o GPCR Receptor Arrays provide a convenient method to analyze compounds against a GPCR family