Automation of Receptor-Ligand Binding Assays using the MultiScreen®$_{HTS}$ Filter Plate

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Abstract

Perhaps the most critical screening parameter in the drug discovery process is the quantification of the specific affinity a drug has for a particular cellular receptor. Whether the process be carried out as a primary screening method for large compound libraries or as a secondary screening tool to rank compounds for binding affinity, high throughput, automation compatibility and accurate receptor-ligand binding analyses are required.

The design features of the new MultiScreen®$_{HTS}$ filter plate allows the plate to be easily gripped and moved around robotic decks and associated stackers. The plate can accommodate standard bar code labeling systems for ease of tracking when processing or storing numerous samples. The MultiScreen®$_{HTS}$ filter plate can be used for coincidence counting which provides the best signal to noise ratio.

The receptor ligand binding data presented here will demonstrate automation of the receptor ligand binding assay using the MultiScreen®$_{HTS}$ filter plate on the PerkinElmer Evolution™ P^3 Workstation. Scintillation counting and analysis were performed on a Wallac Microbeta® TriLux.

MultiScreen®$_{HTS}$ Filter Plate Assay System

- 96 well format designed for highly sensitive and specific radiometric assay
- Removable underdrain for increased radiometric signal
- Completely isolated wells prevent radiometric signal crosstalk
- Completely ANSI-SBS compatible for full automation on robotic workstations
- Rigid side walls allow for easy barcode attachment
- Wide selection of filtration media including glass fiber

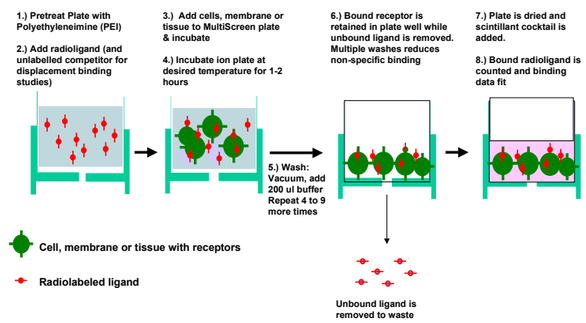
Methods and Materials

MultiScreen® (Millipore, FB glass fiber cat# MAFB N0B or HV membrane cat# MAHV N0B) and MultiScreen®$_{HTS}$ HV (Millipore, FB glass fiber cat# MSFB N0B or HV membrane cat# MSHV N0B) filter plates were pretreated with 0.1% polyethyleneimine (PEI) and washed with appropriate assay buffer.

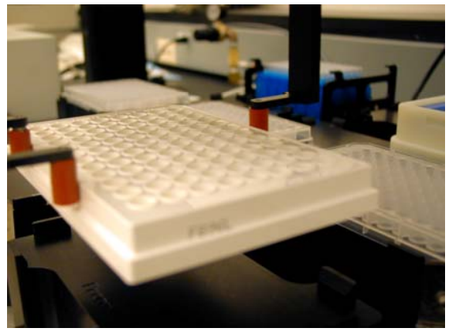
Saturation Binding: 25 ng purified human estrogen β receptor (Sigma) or 8.75 ug of a human Muscarinic M1 receptor expressing transgenic CHO cell membrane fragment bound receptor preparation (PerkinElmer) was incubated with serial dilutions of radiolabeled ligand [^{3}H-estradiol (Sigma) and ^{3}H-scopolamine (PerkinElmer NEN) respectively]. After 1 hour incubation in the filter plate, plates were washed 10 times by successive filling with binding buffer then vacuum. The plates were dried completely before the addition of Opti-Fluor Scintillation cocktail (PerkinElmer cat# 6013199) Non-specific binding was determined in a separate experiment with an excess of unlabeled competitor ligand. Specific binding was calculated as non-specific activity subtracted from total activity. Binding constants (Kd) were determined by fitting specific binding by free ligand concentration by non-linear regression and Scatchard analysis (shown) using Prizm data software (www.Graphpad.com).

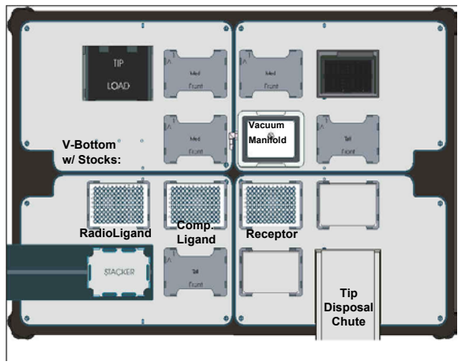
Displacement Binding: Receptor amounts used were the same as for above. Radioligand binding inhibition was determined with a constant radioligand concentration (0.2 nM ^{3}H-estradiol or 0.6nM ^{3}H-scopolamine) and serial dilutions of unlabelled competitor ligand (estradiol and pirenzipine, respectively) as compared to a control binding experiment without unlabelled ligand (% Control). Relative affinity values (IC50) were determined by fitting binding inhibition values by non-linear regression using Prizm data software.

In-Plate Receptor/Ligand Binding in MultiScreen® Filter Binding Plates



Automated Assays with MultiScreen®$_{HTS}$ on PerkinElmer Evolution™ P^3

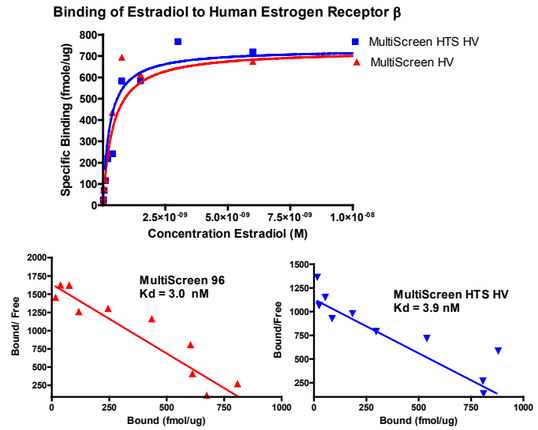




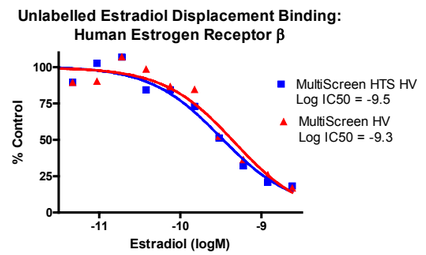
Radioligand, competitive non-radiolabeled ligand and receptor dilution, mixing, and filter plate washing are all performed on the PerkinElmer Evolution™ P^3. The programs allows the user to input number of plates to run, number of filter plate washes, and whether the receptor-ligand binding reaction is performed directly in a filter plate or in a V-Bottom incubation plate followed by transfer to a filter plate

Solublized Nuclear Hormone Receptor Binding on PEI Treated MultiScreen® with HV (0.45μM) Membrane
Comparison of MultiScreen® and MultiScreen®$_{HTS}$

Saturation Binding



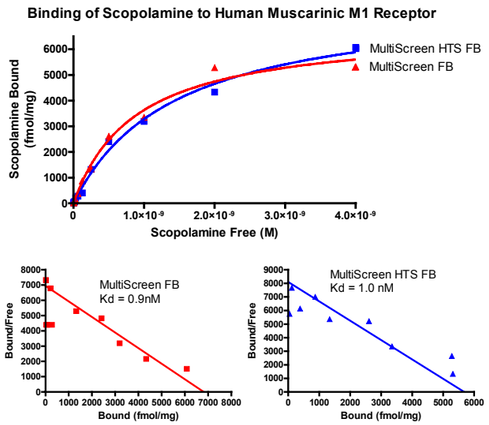
Displacement Binding



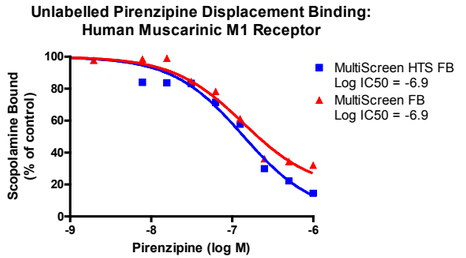
All Data Points in this Figure are the Average of Triplicate Experiments

G-Protein Coupled Receptor (GPCR) Binding on MultiScreen® with PEI Treated FB Glass Fiber
Comparison of MultiScreen® and MultiScreen®$_{HTS}$

Saturation Binding



Displacement Binding



All Data Points in this Figure are the Average of Triplicate Experiments

Summary

•The Millipore MultiScreen®$_{HTS}$ 96 well filter plates with the PerkinElmer Evolution P^3 Workstation allow for easy automation of receptor–ligand binding assays for both quantitative saturation binding and displacement screening experiments.

• Receptor ligand binding assays were performed with a soluble nuclear hormone receptor (the human estrogen β receptor) and a G-protein Coupled Receptor [(GPCR) the human Muscarinic M1 receptor]. Accurate and reproducible determinations of receptor specific activity (Bmax), binding affinity (Kd) and IC50 values of competitor ligands were measured.

•The MultiScreen®$_{HTS}$ filter plate is ANSI/SBS 2004 1-4 standard compliant thus allowing for the use of robotic gripper arms and can accommodate bar code labels.

• The new Multiscreen®$_{HTS}$ filter plate provides the same quantitative, robust and reproducible data acquisition as the original MultiScreen® filter plate platform.

•Receptor-ligand binding reactions can be performed entirely in the filter plate, saving the expense of an extra incubation plate and limiting the number of steps in the protocol for time savings.

•The availability of various filter media allows for the characterization of soluble receptors (e.g. Nuclear Hormone Receptors) and cell membrane embedded GPCRs.