High Density Receptor Ligand Binding Assays in the MultiScreen® HTS 384-well Glass Fiber Filter Plate
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Abstract
Radioisotopic binding assays are acknowledged for their ability to identify and confirm lead compounds in drug discovery. They can be limited in their utility due to throughput, cost, and amenability to automation. Data obtained using a new 384 well filter plate demonstrate that automated quantitative and screening assays can be developed that are equivalent to assays run in a 96-well format.

Using the Muscarinic M1 G-protein Coupled Receptor as a model system, we achieved accurate and reproducible determination of binding affinity (Kd) and IC50 results for known ligands on the 384-well plate device. As a result of being able to incubate the reaction mixture in the filter plate and configure the assay using half the reaction volume, significant reductions in reagent costs and radioactive waste were achieved. Use of the fully-automated 384 filter plate makes it possible to develop higher throughput, lower cost radioisotopic receptor binding assays.

Introduction
Receptors are biological macromolecules located in the cell membranes, the cytoplasm or the cell nucleus that are capable of highly specific binding to chemical ligands. Upon binding of a receptor by a ligand, signal transduction events occur that regulate various biological processes required for proper growth and function of the cell. Many disease states can be treated by regulating the activity of receptors and/or their downstream effectors molecules. For instance, uncontrolled cellular proliferation during cancer can be reduced by blocking the activity of growth factor receptors. Many diseases can be treated by blocking the activity of an endogenous ligand with a more potent chemical entity, either when the production of the ligand is limited or changes in the receptor alter its specificity for the natural ligand. Thus, a major focus in drug discovery is the development of receptor specific agents that can reduce or block inappropriate activity of receptors and/or their downstream effector molecules. For instance, uncontrolled cellular proliferation during cancer can be reduced by blocking the activity of growth factor receptors. In addition to blocking the activity of receptor, many diseases can be treated by replacing the activity of an endogenous ligand with a more potent chemical entity, either when the production of the ligand is limited or changes in the receptor alter its specificity for the natural ligand. Thus, a major focus in drug discovery is the development of receptor specific agents that can reduce or block inappropriate activity of receptors and/or their downstream effector molecules. For instance, uncontrolled cellular proliferation during cancer can be reduced by blocking the activity of growth factor receptors.

Methods
MultiScreen® (Millipore, FB glass fiber cell WAFF NBE) and MultiScreen® 384 (FB glass fiber) filter plates were prepared from unpurified tissue homogenates or cell membrane fragments. To date, most receptor filter binding assays have been performed on 96-well platforms. However, a bottleneck has been performed on 96-well platforms. However, a bottleneck has been performed on 96-well platforms. However, a bottleneck has been performed on 96-well platforms. However, a bottleneck has been performed on 96-well platforms. However, a bottleneck has been performed on 96-well platforms. However, a bottleneck has been performed on 96-well platforms. However, a bottleneck

Saturation Binding: Unless otherwise stated, all binding experiments were performed by mixing the reagents in the filter plate and incubation to equilibrium was performed directly in the plate as well. B.T. pg (96 well) or 4.3 pg (384 well) of a human Muscarinic M1 receptor expressing transgenic CHO cell membranes or transgenic CHO cell membranes fragment were added to the plate. Scopolamine (PerkinElmer) was immobilized (200 pg/well) or 100 pg (384 well) with serial dilutions of radiolabeled Pirenzepine (PerkinElmer NEN). After 1 hour incubation in the filter plate, plates were washed 10 times with binding buffer then vacuum. The plates were dried completely before the addition of Opti-Fluor Scintillation cocktail (PerkinElmer cat# 6013199). Scintillation counting and analysis was performed on a Wallac Microbeta Tri-Lac scintillation counter in default coincidence counting mode. Specific binding was determined in a separate experiment with an excess of unlabeled competitor ligand (pirenzepine (Pir). Specific binding was calculated as non-specific activity subtracted from total activity. Binding constants (Kd) were determined by fitting specific binding to non-linear regression and Scatchard analysis (shown) using Prism data software (Graphpad.com).

Reproducibility of Binding Parameter Determination On MultiScreen HTS Plate

Receptor-ligand binding assays can be performed in a 384 well filter plate format with the same robustness and reproducibility as 96 well filter plate formats.

• Receptor-ligand binding assays were performed with a G-protein Coupled Receptor (GPCR), the human Muscarinic M1 receptor. Accurate and reproducible determinations of receptor specificity (Bmax), binding affinity (Kd) and IC50 values of competitor ligands were measured. Values obtained in 96 well and 384 well FB glass fiber plates were in agreement with each other and literature values.

• By optimizing the 384 well filter plate for coincidence counting, highly quantitative data can be collected at very low levels of radioactivity over a large range.

• Receptor-ligand binding in the 384 well filter plate was performed with half the reagents in half the assay volume as the 96 well format.

• Cost savings through the use of less reagents can be achieved without sacrificing sensitivity.

• Receptor-ligand binding assays can be performed directly in the filter plate (“in-plate”) decreasing the number of manipulations, and reducing radioactive waste by eliminating the need for a separate incubation plate.

• The of 384 well filter plate can be used to screen large libraries of compounds in a high density platform.


Summary

View of MultiScreen® 384 well Filter Binding Plate on PerkinElmer Evolution® P3 Platform

G-Protein Coupled Receptor (GPCR) Binding on MultiScreen® Plate with PEI Treated FB Glass Fiber

Schematic of In-Plate Receptor/Ligand Binding in MultiScreen Glass Fiber Filter Binding Plates

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