

Automation of Radiometric Filter-Based Phosphorylation Assays Using Positive Pressure

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Abstract

A major focus in drug discovery involves the identification of compounds that reduce or modulate biological processes by altering the activity of cellular receptors or signal transduction components such as kinases. Heterogeneous filter binding assays have been successfully used to screen many compounds for potential drug activity. Filter-based assays have the advantage over homogeneous formats in that multiple washing of the filter greatly improves signal to background by removing non-specific binding.

The recent availability of 384-well filter plates and improved automation capabilities have expanded the utility of heterogeneous binding assays allowing for higher throughput and reduced reagent costs. Where filter-based assays have traditionally used vacuum as a means of separation, positive pressure systems are another effective method of separation which are very amenable to automation.

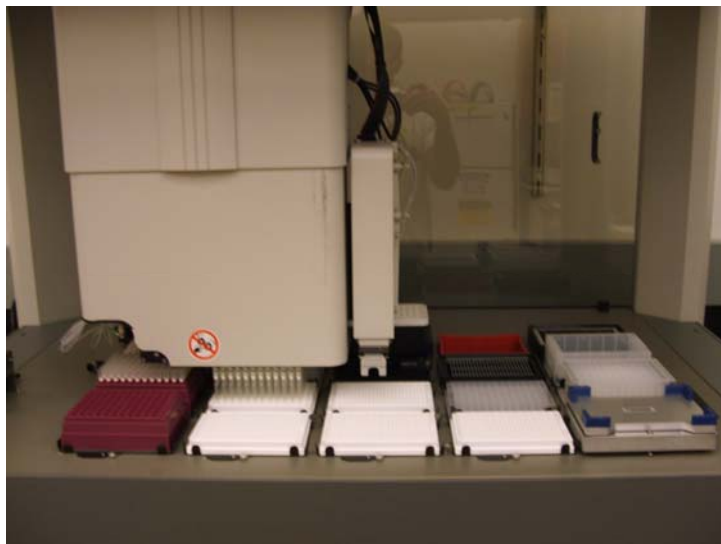
The performance of the Millipore MultiScreen[®]_{HTS} 384-well filter plate is demonstrated using an automated radiometric assay for screening compounds on Caliper's Sciclone[™] ALH 3000 Workstation with a positive pressure filtration accessory. Protein Kinase A was used as the representative assay system with which we were able to screen a number of different compounds. Z' values were measured showing the integration of automation and the 384-well format.

Introduction

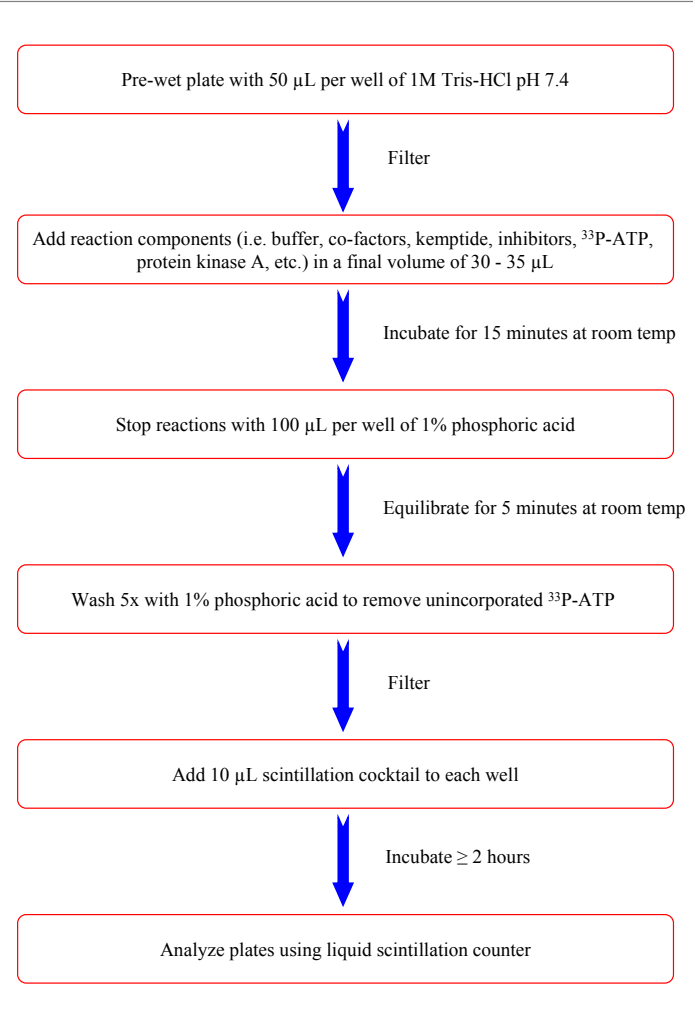
High throughput screening using phosphocellulose (PH) filters is a proven methodology used to identify kinase inhibitors and has been well-documented with the MultiScreen 96-well filter plate. The ability of PH paper to retain phosphorylated peptides via ion-exchange, while allowing for the passage of unincorporated ³³P-ATP, is a central element of this filter binding assay. This system allows for the direct measurement of phosphorylation with superior sensitivity. With the advent of the MultiScreen 384-well format, which shares the same benefits as the 96-well plate, an increase in throughput is realized in addition to being able to perform the reaction setup directly within the filter plate.

The Sciclone ALH 3000 workstation with options such as the Gripper, Twister[®] II Plate Handler, and the integrated "swap-on-the-fly" pipetting head exchange technology allows for multiple plate processing and high throughput screening capabilities. The Positive Pressure Filtration System provides for consistent well-to-well evacuation and is controlled directly through the software. Positive pressure provides many advantages in filter-based assays, including reliable self-sealing integrity.

Kinase Assay using MultiScreen_{HTS} 384-well PH (MZPH) Filter Plates on the Sciclone ALH 3000 Workstation



Method

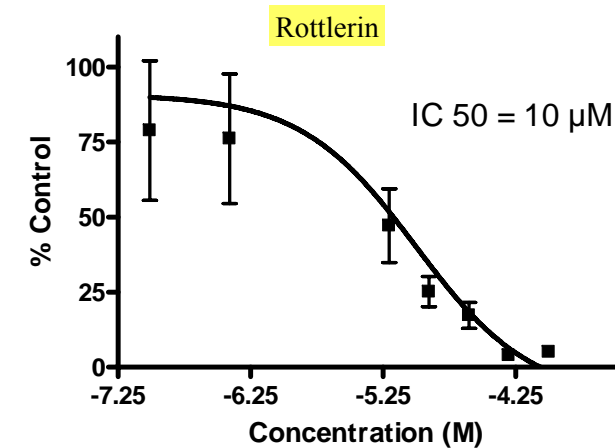
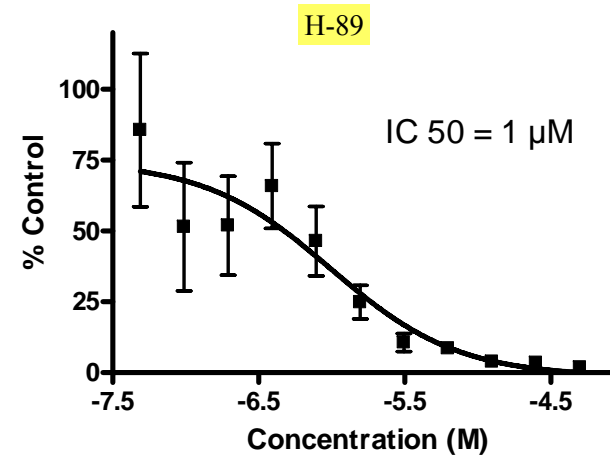
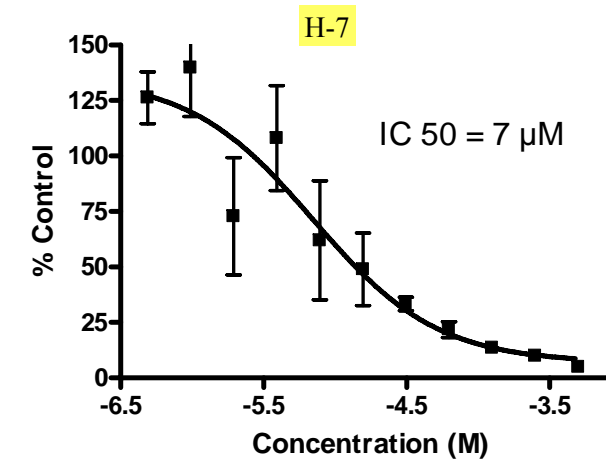


Positive Pressure Filtration System



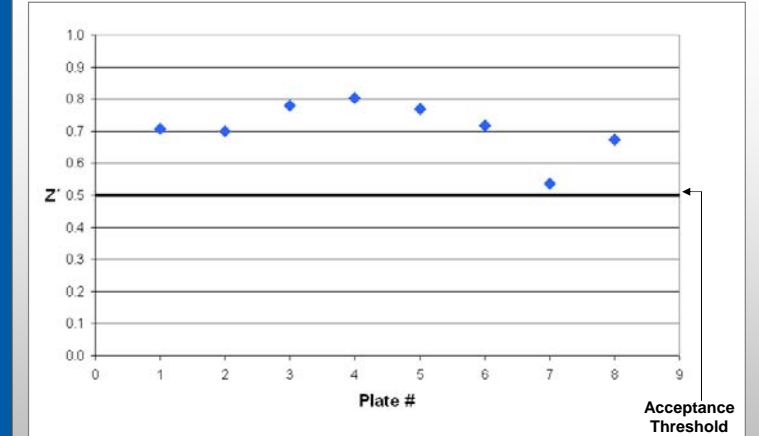
The Caliper Positive Pressure Filtration System is commonly used in automated filter plate assays in place of a vacuum filtration system and has been demonstrated with solid phase extraction for several years. Positive pressure allows for a known, reproducible pressure to be applied to the filter plate and this equates to an easy method transfer between labs. The Positive Pressure head offers self-sealing integrity by reliably forming a seal on top of the filter plate resulting in confidence during filtration steps.

Compound Screening via IC₅₀ Analysis



IC₅₀ determination was carried out using enzyme and substrate-limited inhibition reactions across a concentration range of the compounds. These were carried out directly within the MultiScreen 384-well PH plate. Reactions were initiated then terminated at 15 minutes in synchrony. Data points represent replicates of four. IC₅₀ values were calculated by non-linear regression plotting of the normalized corrected CPM data versus compound concentration using GraphPad Prism[™] 4 Software (www.graphpad.com).

Z' Evaluation of Kinase Screening Assays



30 µL phosphorylation reactions of kemptide by protein kinase A were run in adjacent rows of the MZPH filter plates in the presence or absence of the kinase inhibitor piceatannol (300µM). Each data point represents an individual plate (48 sample wells). Z' analysis is a statistical measure used to evaluate assay robustness and quality. Z' values were determined by the formula $Z' = 1 - ((3s_u + 3s_i) / (m_u - m_i))$ where s = standard deviation, m = mean, u = uninhibited and i = inhibited¹.

Summary

- * Satisfactory IC₅₀ curves were generated from the screening assay for multiple compounds using the MultiScreen_{HTS} 384-well PH filter plate with the Sciclone ALH 3000 workstation. Curves are comparable to those generated using vac filtration (data not shown).
- * By eliminating the need for a separate incubation plate and an additional transfer step, it was possible to further streamline the process and cut down on the cost and time of the assay.
- * Calculated Z' values demonstrate that this phosphocellulose based methodology is well suited for high throughput screening using the Sciclone workstation. Note: Z' = 0.5 suggests that the number of potential false negatives will be less than 1 per a compound library of 1 billion.
- * The Sciclone ALH 3000 and Positive Pressure Filtration System generates results that confirm the suitability of radiometric filter-based assays using automation for high throughput screening.

References

- 1.) Zhang, J., Chung, T., and Oldenburg, K., J. Biomolecular Screening 4:67-73, 1999