

Nano high-throughput screening (nHTS) platform - miniaturization of cell-based GPCR and kinase assays

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- Cell-based, nanoHTS platform can screen as little as 250 cells/well in a volume less than one microliter
- PathHunter® GPCR and Kinase cell-lines were dispensed using the Echo 555 liquid dispenser
- PHERAstar® FS was used for detecting the signal in 1536- and 3456-well microplates

Introduction

With the development of more sensitive technologies, further miniaturization of cell-based assays for high-throughput screening is now very feasible. Performing cell-based assays in sub microliter volumes, translates into very low compound requirement and fewer cells used. In addition to being more economical, more experiments can be done in the same amount of time and at the same overall price.

Combining technologies from three companies, an nHTS platform was developed to screen GPCRs (shown here) and kinases (data not shown here) in a cell-based assay format in 1536 and 3456-well plates. Technologies used include DiscoverX's PathHunter® GPCR beta-Arrestin and PathHunter® Cell-based Kinase assay platforms; Labcyte's Echo® liquid handling system to transfer cells and compounds; and BMG LABTECH's PHERAstar® FS microplate reader for low volume detection.

Materials & Methods

- PHERAstar® FS from BMG LABTECH
- Echo® 555 liquid handler from Labcyte
- PathHunter® cell lines and assay reagents from DiscoverX
- 1536- and 3456-well microplates from Aurora

Cell Handling

PathHunter® Cells were diluted in Hank's Buffered Saline Solution. 40 µL were added to Echo® qualified 384-well polypropylene microplates (P-05525). PathHunter® Cells were transferred to an Aurora 1536-well or 3456-well microplate using an Echo® 555 liquid handler with a 384PP_AQ_SP calibration. Cell volumes were varied as part of the testing plan. Compounds such as pergolide, ProLactin or β-NGF were diluted in DMSO and added to an Echo® qualified 384-well polypropylene microplate and transferred at 30 nL using the 384PP_DMSO calibration. Incubation as per β-arrestin and Cell-based Kinase Assay recommendations.

Addition of Detection Reagents

PathHunter® Flash Detection reagent was transferred from an Echo® qualified 384-well microplate and transferred (at a 1:1 ratio of cell volume: PathHunter® Flash Detection reagent) with the Echo® 555 liquid handler with the 384PP_AQ_SP calibration. Incubate for 30 mins at RT.

Detection

Both 1536- and 3456-well microplates were measured on the PHERAstar® FS using a measurement time of 0.1 sec/well at a gain of 3600 with a luminescence optic module. An automatic z-height adjustment was performed on the well with the highest signal before reading the plate. The 0.1 mm adjustable z-height allows the PHERAstar® FS to obtain the highest possible signal-to-noise ratios in all cell-based assays.

Results & Discussion

Adherent Format

PathHunter® U20S cells expressing Trk A and PRLR-JAK1 at 1000 cells/well were seeded in a white TC coated AURORA 1536 plates and stimulated with known agonists β-NGF and prolactin. These plates were incubated for 3 hrs at 22°C. A robust assay response is measured with 6 µL total volume (Figure 2).

Assay Principle

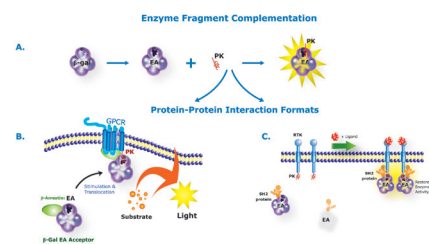


Fig. 1: PathHunter® assay principle.

The PathHunter® technology from DiscoverX is an adaptation of EFC that provides a novel cell-based assay format for detecting protein-protein interaction. In this approach, ProLink™ is appended to GPCR (1B) or RTK or cytokine receptor (1C), while EA is recombinantly expressed as a fusion protein with β-Arrestin (Fig 1B) or SH2 phosphotyrosine domain (Fig. 1C). Activation of the receptor upon ligand binding allows interaction of GPCR-β-Arrestin or RTK-SH2 protein resulting in a measurable chemiluminescent signal.



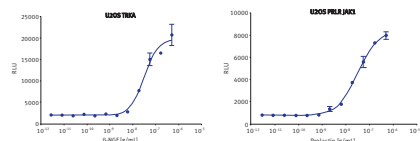


Fig. 2: PathHunter® U2OS cell receptor tyrosine kinase assay in a 1536-well adherent format stimulate with prolactin (left graph) and β -NGF (right graph) using 1000 cells/well.

PathHunter® CHO-K1 cells expressing Long isoform of Dopamine receptor D2 were seeded in a white TC coated AURORA 1536 plates at 1000 cells/well and incubated overnight for cells to adhere. Cells were stimulated with Pergolide for 90 minutes at 37°C (figure 3).

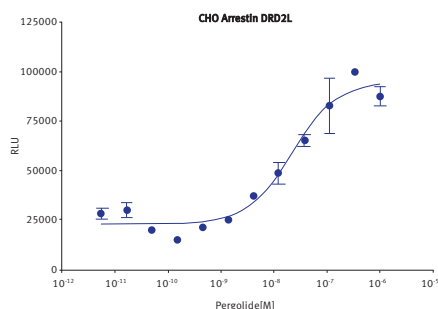


Fig. 3: Adherent CHO cells with PathHunter® β -Arrestin Assay measuring the Dopamine 2L receptor (DRD2L) in a 1536-well using 1000 cells/well.

These results indicate that the PathHunter® arrestin (and kinase) assay can be successfully adapted to detection in 1536 well microtiter plates using adherent cells.

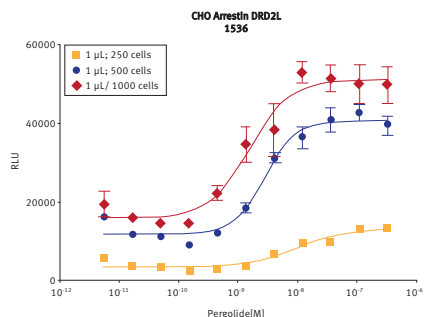


Fig. 4: Cell-seeding assay. PathHunter® DRD2L cells from 250 to 1000 cells/well in suspension were added to 1536 well plates in a 1 μ L volume and stimulated with 5-10 nL of 100X pergolide (in DMSO) added to cells via the Echo® Liquid handler.

Different suspensions of CHO cells are shown in figure 4, the DRD2L 1536 suspension protocol produced dose curves similar to the adherent cell experiment. For optimal performance it is recommended that cell seeding volume using the Echo® instrument not exceed 1 μ L.

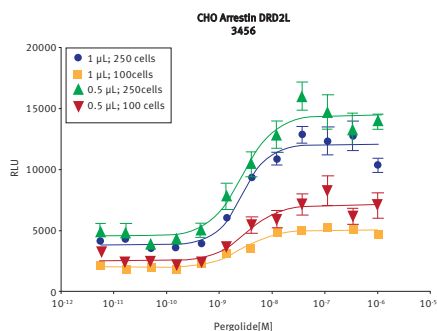
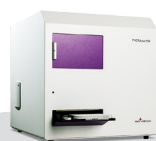


Fig. 5: 3456-well Protocol Optimization. PathHunter® DRD2L cells 100 or 250 cells/well (in suspension) were added to the AURORA 3456 well plates in 0.5 or 1 μ L volumes via the Echo® instrument. Compound [5nl of 100X in DMSO] was added to the cells plated in 0.5 μ L (10 nl for the cells plated in 1 μ L) via the Echo® instrument.

The protocol for cell seeding using the Echo® 555 was applied to cell dispensing into 3456 well plates, figure 5. The performance of different cell densities was tested to determine the optimal cell number in the new plate format. PathHunter® DRD2L cells 100 or 250 cells/well (in suspension) were added to the AURORA 3456 well plates in 0.5 or 1 μ L volumes via the Echo® instrument. Compound [5nl of 100X in DMSO] was added to the cells plated in 0.5 μ L (10 nl for the cells plated in 1 μ L) via the Echo® instrument.

Conclusion

Miniaturization with highly evolved automation technologies affords the benefit of faster screens at much lower costs. Here we have demonstrated the application of the DiscoverX's PathHunter GPCR and cell-based kinase assays in 3456 and 1536 platforms using Labcyte's ECHO Liquid handler and BMG LABTECH's PHERAstar FS. We describe an nHTS cell-based screening platform that can measure as little at 250 cells per well using less than 1 microliter.



PHERAstar® FSX

*The PHERAstar FSX is the newest PHERAstar reader.