Functional Receptor Tyrosine Kinase Assays for Antibody Therapeutics Using PathHunter™ Technology

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Abstract

Kinase targets have been extensively studied in biochemical assays using purified protein fragments for the kinase and the substrate. However, there is an increasing need to understand how kinases function in the context of a whole cell assay. DiscoveRx has pioneered a protein interaction detection system using well-established Enzyme Fragment Complementation (EFC) technology, by monitoring the interaction of target proteins with SH2 phosphotyrosine binding domains. We have successfully applied this approach to multiple receptor tyrosine families. Over the recent past, monoclonal antibodies have become a growing class of potential drugs for cancer and other therapeutic areas. In this study, we demonstrate the application of the PathHunter cell-based kinase assays to detect anti-receptor and anti-ligand antibody that function specifically against EGFR, c-Met, TrkA. This data demonstrates that PathHunter™ Cell-Based kinase assays provide a robust, sensitive and easy-to-use method for identifying novel antibodies in a whole cell, homogenous assay that is also high-throughput friendly.

PathHunter™ Assay Principle

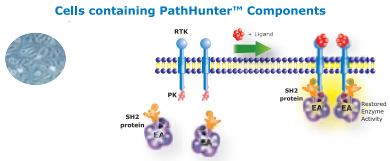


Figure 1. The PathHunter™ Tyrosine Phosphorylation assay monitors the interaction of tyrosine phosphorylated proteins with SH2 containing proteins in a whole cell, homogeneous assay format using enzyme fragment complementation. In this system, a small 42 AA enzyme fragment, ProLink is appended to the C-terminus of the receptor target. The SH2 protein is fused to the larger enzyme fragment, EA (Enzyme Acceptor). Activation of the receptor initiates tyrosine phosphorylation and binding to the SH2-EA fusion that forces complementation of the two enzyme fragments. This action results in the formation of fully complemented β-galactosidase enzyme, the activity of which is measured using chemiluminescent PathHunter detection reagents.

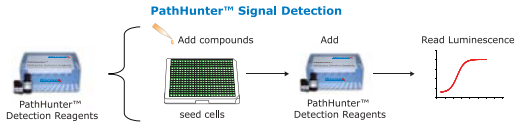
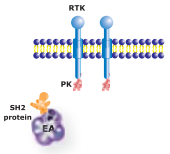


Figure 2. The PathHunter™ Detection Kit consist of a lysis buffer and substrate mixture that is added as a single solution to stimulated cells. After a short incubation, the PathHunter chemiluminescent signal can be detected using any standard luminometric plate reader.

Benefits of PathHunter™ Receptor Tyrosine Kinase Assays



Assay Attribute	Advantages
Full length Receptor	• Identify Anti-receptor antibodies • Identify Anti-ligand antibodies • Identify Activating antibodies • Identify Non-ATP Pocket Binders • Identify Ligand Binding inhibitors • Indentify Dimerization inhibitors
Whole Cell Assay	• Get cell permeability information
Single Addition Assay	• High-throughput friendly format

Assay measures ligand binding, phosphorylation and signal transduction. It measures the interaction between receptor tyrosine kinases and adaptor proteins such as SHC, GrB2, PLCG1, PLCG2 or P85.

Simple One-step Assay That Detects Agonists or Partial Agonists

Figure 3. Left panel, in PathHunter™ PDGFRb assay, like most receptor tyrosine kinases PDGFRb homodimerizes in the presence of an appropriate agonist, PDGF-AB causes recruitment of SH2 proteins and can be monitored using the PathHunter technology. PathHunter cells expressing PDGFRb and Neurotrophin receptor Trk A were incubated with increasing concentrations of the respective agonist/Partial agonist. PathHunter™ Detection Kit (93-0001) was used in this assay. Assays were run in a 384-well plate and read on a standard luminometer. The right panel shows PathHunter™ TrkA assay can differentiate **full and partial agonists.**

Detect Small Molecule Inhibitors

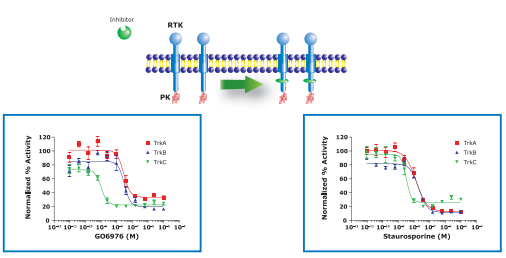


Figure 4. PathHunter™ cells expressing neurotrophin receptors Trk A, Trk B, Trk C were incubated with increasing concentrations of the small molecule inhibitor G06976 and staurosporine. Both are Protein Kinase C inhibitors. PathHunter™ Detection Kit (93-0001) was used in this assay. Assays were run in 384-well plate and read on a standard luminometer.

Novel Assay Platform for Antibody Therapeutics

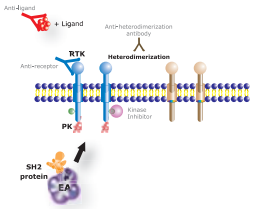


Figure 5. Monoclonal antibodies have been extensively used in cancer treatments. The figure above illustrates the capabilities of PathHunter cell-based kinase assays in identifying different types of antibodies against a receptor tyrosine kinase of choice.

Detect Anti-Ligand Antibody

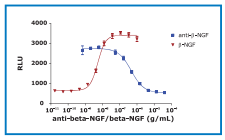


Figure 6. U2OS TrkA cells were plated in a 384-well plate at the density of 5,000 cells/well in cell plating media and grown overnight in a 37°C incubator. Anti-human β-NGF Ab was diluted sequentially to make 5X working solutions. 5μL antibody and then 5 μL β-NGF per well were added. PathHunter™ Detection Reagent (93-0001) was used in this assay. Assays were run in a 384-well plate and read on a standard luminometer. β-NGF shows an agonist response, while anti-β-NGF antibody shows an inhibition in this assay.

TrkA Activating Antibodies

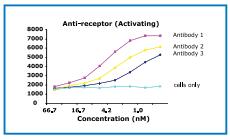


Figure 7. In this experiment TrkA cells were used to screen 3 antibodies that have the ability to activate TrkA receptor in the absence of ligand. The experiment was performed by incubating TrkA cells with increasing amounts of the activating antibodies to determine which antibody had the greatest effect on TrkA receptor activation.

Anti-Receptor Blocking Antibodies: EGFR and c-MET Blocking Antibodies

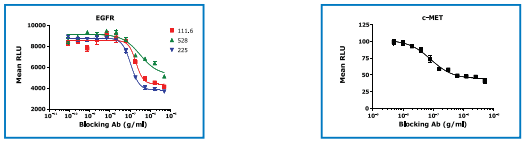


Figure 8. In this experiment EGFR and c-MET cells were used to screen commercially available antibodies that have the ability to block EGFR and c-MET. The experiment was performed by incubating EGFR and c-MET cells with increasing amounts of the blocking antibodies to determine which antibody had the greatest inhibitory effect.

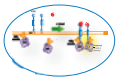
Summary

PathHunter cell-based kinase assays provide distinct advantages over traditional biochemical kinase screening

- **One step, chemiluminescent, whole cell assay**
- **Perform kinase screens under physiologically relevant ATP and substrate concentrations**
- **Identify novel class of kinase inhibitors and not just ATP pocket binders**
- **Involve both small molecule and/or antibody libraries in your screening campaign**
- **Identify both anti-ligand or anti-receptor antibodies**

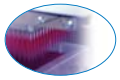
In this presentation we have demonstrated the application of the PathHunter Receptor Tyrosine assays to screen and detect various antibody functions, from receptor activation to ligand inhibition. The data shows that not only is the PathHunter platform capable of detecting such activity, it also shows that the assay can also be used as an efficient screening tool to identify potent antibodies.

Technology Access



PathHunter™ Cell-Based Kinase Assays
Clonal cell lines expressing ProLink-tagged Receptor Tyrosine Kinase on the membrane and EA-SH2 fusion protein in the cytoplasm. Over 15 cell lines are now available. For more information, please visit www.discoverx.com/kinases.

Custom Projects
Utilize DiscoveRx's proprietary EFC technology to build your own functional cell-based kinase assays. Talk to our experts about your kinase targets, contact CAD@discoverx.com.



Custom Screening & Profiling Services
Send your compound for simple profiling, specificity studies or selectivity profiling against one or many receptor tyrosine kinase targets. For more information, please email profiling@discoverx.com.

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