A Wide Range of Compound Efficacies Revealed Using PathHunter™ Arrestin Assays

Rocco Raduazo*, Qumber Jafri, Mong Saetern, Judy Leon, Daniel Bassoni, Philip Achacoso, William Raab and Tom Wehrman, PhD

DiscoveRx Corporation, Fremont, CA 94538, USA

Abstract

DiscoveRx has developed a novel application of our established **E**nzyme **F**ragment **C**omplementation (EFC) technology that allows us to monitor protein-protein interaction events in whole cells. Using this approach, we have designed the PathHunter™ Arrestin assay that monitors arrestin binding to activated GPCRs. The activity is subsequently detected with a single addition, HTS friendly, chemiluminescent read out. β-Arrestin recruitment is a well established aspect of GPCR biology, and DiscoveRx has now successfully commercialized over 220 different cell lines for unique GPCR targets analyzed with this approach. In accord with natural biological functions in cells, the PathHunter Arrestin assays measure the activation of receptors in a stoichiometric, one-to-one fashion, where receptor activation is reflected in a linear increase in chemiluminescent signal. This is in stark contrast to other functional assays that are amplified via signal transduction cascades where the maximum signal is dictated by rate-limiting endogenous cellular proteins. This novel property of the PathHunter Arrestin assay makes the pharmacology independent of receptor expression levels and provides an ideal platform for accurate efficacy measurements of both agonists and partial agonists.

PathHunter β-Arrestin GPCR Assay System

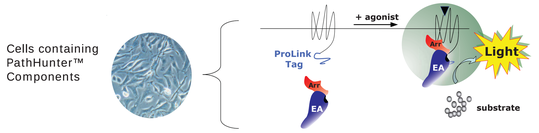


Figure 1. PathHunter β-Arrestin Assays monitor the interaction of β-Arrestin with activated GPCRs using Enzyme fragment Complementation (EFC). In this system, a small 42 AA enzyme fragment (called ProLink™) is appended to the C-terminus of the GPCR. Arrestin is fused to the larger enzyme fragment (called EA or Enzyme Acceptor). Activation of the GPCR stimulates binding of Arrestin and forces complementation of the two enzyme fragments, resulting in an increase in enzyme activity that is measured using chemiluminescent PathHunter Detection Reagents.

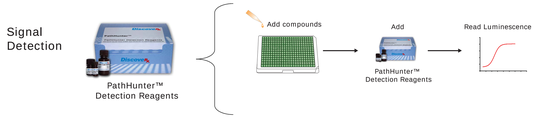
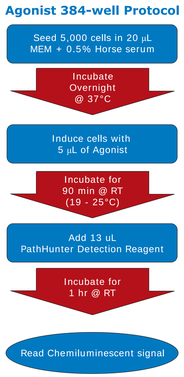


Figure 2. The PathHunter Detection Reagents consist of a lysis buffer and substrate mixture that is added as a single solution to stimulated cells. After a short incubation, the PathHunter chemiluminescent signal can be detected using any standard benchtop luminometer.

Methods



Pharmacology is Independent of Receptor Expression Levels

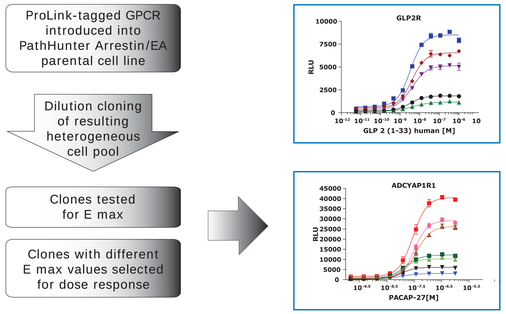


Figure 3. The PathHunter Arrestin Assay is a stoichiometric system where signal is generated from each activated receptor. This system is independent of intracellular systems and thus should operate at zero receptor reserve levels. To illustrate this point, clones were isolated with varying levels of E max from GLP2R and ADCYAP1R1 (PAC1) heterogeneous pools and tested in dose response format with their respective full agonists. As shown above, the EC_{50} values of each clone (denoted by a distinctly colored line) were similar across a wide range of expression levels.

Assay Performance of the PathHunter System

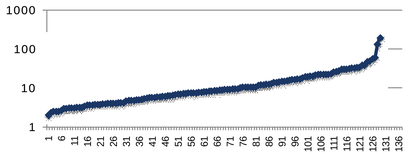


Figure 4. Signal to background (S:B) ratios were measured on 147 PathHunter Arrestin clones each expressing a unique receptor using an optimal concentration of full agonists. The receptors are plotted along the X-axis with their corresponding S:B ratios plotted on the Y-axis. These results show that over half of all targets exhibited more than a 10-fold signal-to-noise ratio which makes this assay ideal for efficacy measurements especially for difficult targets such as Gi- and Go-coupled receptors.

Compound Efficacy of the ADRB2 Receptor

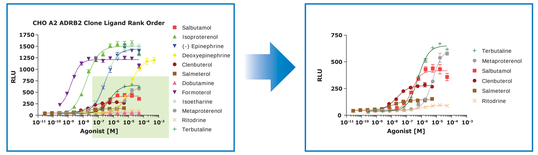


Figure 5 . The PathHunter Arrestin clone expressing the β2 Adrenergic Receptor (ADRB2)-ProLink fusion was tested for agonist responses to a variety of full and partial agonists. These results indicate a wide range of compound efficacies for each of these compounds. The lower graph depicts the compounds in the shaded box. In this graph, the full agonists were removed to provide a better visualization of the efficacies. Notably all partial agonists were detected as partial agonists.

Detection of "Super" Agonists

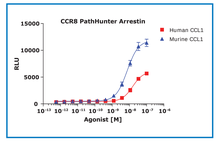


Figure 6. Many full agonists are characterized by second messenger signaling assays that may suffer from masking full efficacies due to receptor over expression and receptor reserve. In this experiment, treatment of the Human CCR8 PathHunter Arrestin clone with the murine CCL1 (mCCL1) ligand was shown to exhibit a higher efficacy than the human CCL1 (hCCL1) ligand indicating that the mCCL1 is a "super" agonist at the human CCR8 receptor.

Summary

The PathHunter β-Arrestin system utilizes arrestin binding to activated GPCRs to generate an enzymatic signal. This mode of detection is not limited by the cellular machinery and detects agonism at every cell-surface exposed receptor. This property, in combination with the large signal-to-noise ratios obtained with the system, provides an ideal screening platform and enhanced efficacy measurements of lead compounds.

In this study, we show that the PathHunter system is able to detect a range of efficacies at the ADRB2 receptor with the appropriate rank order potency of agonists and a wide range of efficacies making it ideal for partial agonist characterization. Further, the stoichiometric signal generation inherent with the system is able to readily detect "super" agonists as shown with the murine CCL1 ligand and the human CCR8 receptor.

Technology Access Section

Cell Lines

Finished cell lines containing the Arrestin-EA and GPCR-ProLink fusions as heterogeneous pools or clonal cell lines. More than 150 cell lines available.

Tool Box

A clonal PathHunter parental cell line and a ProLink vector are available for build-your own cell lines (7 cell backgrounds available)

Kits

Cells, detection reagents, and plates ready-to-go right out of the freezer. Each kit contains enough cells for 2 X 96 well plates

Discovery Partnerships

Custom screening and profiling against PathHunter GPCRs

Contact Information

Offices

United States

DiscoveRx Corporation
42501 Albrae Street
Fremont, CA 94538
United States

To place an order:
t | 510.979.1415
f | 510.979.1650
e | info@discoverx.com
toll-free | 866.448.4864
www.discoverx.com

European Regional Headquarters

DiscoveRx Corporation Ltd. (United Kingdom)
Faraday Wharf, Holt Street
Birmingham Science Park Aston
Birmingham, B7 4BB
United Kingdom

To place an order:
t | +44.121.260.6142
f | +44.121.260.6143
e | info@discoverx.com
www.discoverx.com

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DiscoveRx
www.discoverx.com