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

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Abstract
DiscoveRx has developed a novel application of our established Bioassay® Fragment Complementation (SFC) 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 quantitated with a single addition, VTS friendly, chemiluminescent read-out. A critical aspect of this assay is that it is independent of receptor expression levels and provides an ideal platform for accurate pharmacology studies. This is in stark contrast to other functional assays that are amplified via signal transduction events in whole cells. Using this approach, we have designed the PathHunter™ Arrestin Assay System.

Methods

1. Add compounds to the PathHunter™ Arrestin assay.
2. Induce cells with 90 min @ RT.
3. Incubate for 1 hr @ RT.
4. Seed 5,000 cells in 20 μL of agonist.
5. Read Chemiluminescent signal.

Figure 3. 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.

Figure 4. The PathHunter Detection Reagents are based on the principle that complementation of two enzyme fragments, resulting in an increase in enzyme activity that is measured using chemiluminescent PathHunter Detection Reagents.

Figure 5. GLP-2 (1-33) human [M] is introduced into the human CCR8 cell pool for E max values selected.

Figure 6. Signaling in background (BG) values were measured as 100% PathHunter Arrestin clones showed non-competing or unique agonist activity as optimal concentration (EC50) agonists. The responses are plotted against the line with the corresponding EC50 other agonists plotted on the Y axis. Each line represents the specific IC50 values (EC50) agonists were plotted as agonists that were identified as partial agonists.

Figure 7. The PathHunter Arrestin assay expressing the β2AR Arrestin (ARR2) pathway was induced with agonist followed by a variety of β2AR agonists. These results identify a wide range of compound efficacies for each of these compounds. The lower graph depicts the IC50 values for the compounds that make up the dose response graph.

Figure 8. Pharmacology is Independent of Receptor Expression Levels. The PathHunter™ Arrestin Assay System is able to readily detect “super” agonists. This property, in combination with the large signal-to-noise ratios obtained with the system, provide ideal screening platforms for pharmacological measurement.

Summary
The PathHunter™ 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 simply reports agonist potency and receptor expression. This property, in combination with the large signal-to-noise ratios obtained with the system, provide ideal screening platforms for pharmacological measurement.

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