

Rapid Quantification of Bioactive Ligands in Serum and Human Plasma Using PathHunter® eXpress β -Arrestin GPCR Assays

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- Universal technology used to detect biomarkers for cancer, inflammation, metabolic disorders, diabetes, and many other disease states
- Robust quantification of functionally active ligands in complex biological samples (e.g., serum, blood plasma, or tissue culture supernatants)
- Simple mix-and-read protocol for rapid and reliable results, every time
- Off-the-shelf kits for convenient, routine testing of protein biomarkers

Offering the convenience of a simple, ready-to-use, complete kit, DiscoverX's **PathHunter® eXpress β -Arrestin GPCR Assays** utilize the most rapid and reliable method for quantifying bioactive peptides. With their unparalleled sensitivity, specificity, and ease of use, PathHunter eXpress kits provide the best *in vitro* quantitative tool for discovery and analysis of protein biomarkers found in complex biological samples.

Why Use a Functional, Whole Cell GPCR Assay For Biomarker Detection?

Biomarkers are becoming an essential part of clinical GPCR drug discovery programs as indicators of drug efficacy and predictability of potentially failed compounds. Identification and detection of functionally active biomarkers can accelerate the overall drug discovery process. Many naturally occurring bioactive peptides (Glucagon and Ghrelin), hormones (Glucagon-Like Peptide-1, GLP-1), and chemokines bind to and activate GPCR receptors and have already been associated with specific disease states. Furthermore, cleaved fragments that result from intrinsic proteolytic processing in biological samples can show increased activity (e.g., CCR1 ligands), act as antagonists or become functionally inactive.

While classical methods of biomarker detection, such as mass spectrometry and immunoassays, can both detect and quantitate biomarkers, they cannot differentiate between biologically active and inactive fragments. Therefore, there is a need for a simple, convenient and reliable assay platform that is compatible with complex biological samples and can help accelerate clinical validation of therapeutic candidates.

How Do PathHunter® eXpress β -Arrestin GPCR Assays Work?

PathHunter eXpress assays are based on the proven PathHunter β -Arrestin technology that monitors GPCR activity by measuring the interaction of Arrestin with the activated GPCR using β -galactosidase (β -gal) enzyme fragment complementation (EFC; Fig. 1). In this system, the GPCR of interest is fused in frame with the small, 42 amino acid fragment of β -gal (called ProLink™) and co-expressed in cells stably expressing a fusion protein of β -Arrestin and the larger, N-terminal deletion mutant of β -gal (called enzyme acceptor or EA). Activation of the GPCR stimulates binding of β -Arrestin to the ProLink-tagged GPCR and forces complementation of the two enzyme fragments, resulting in the formation of an active β -gal enzyme. This action leads to an increase in enzyme activity that can be measured using chemiluminescent PathHunter® Detection Reagents.

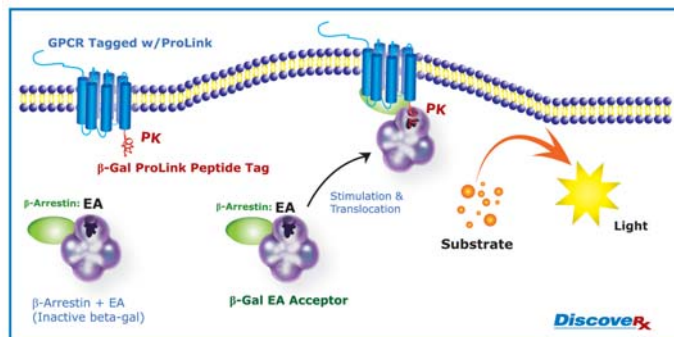


Figure 1. Biological Principle of PathHunter® β -Arrestin GPCR Assay

Convenient, Complete Kit Format

PathHunter eXpress β -Arrestin assays are packaged as convenient, ready-to-use kits containing all components needed to run a live-cell GPCR experiment. Kits include single-use, assay-ready, frozen PathHunter eXpress cells expressing the GPCR target of interest, optimized cell culture (OCC) media, chemiluminescent detection reagents, and 96-well plates. Washing steps are not required. Because accurate, reproducible results on functional biomarkers are obtained quickly using standard luminometers, PathHunter eXpress β -Arrestin assays can reduce the cost of biomarker screening.

Simple Mix-and-Read Chemiluminescent Protocol

PathHunter eXpress GPCR Assays are fully validated for optimal assay performance. Simply thaw the single use frozen cell vials, dilute the cells in the provided Optimized Cell Culture (OCC) medium, and plate cells at 10,000 cells per well in 96-well plates. Ready-to-assay, frozen cells, combined with a simple, one-step addition protocol and standard chemiluminescent detection reagents allow you to get results fast (Fig. 2). As the chemiluminescent signal is the result of GPCR activation, the data directly correlates with the ability of a biomarker to affect GPCR signaling. Moreover, chemiluminescent detection provides superior sensitivity, due to the high ratio of signal to background, and convenience of measurement using any standard benchtop luminometer.

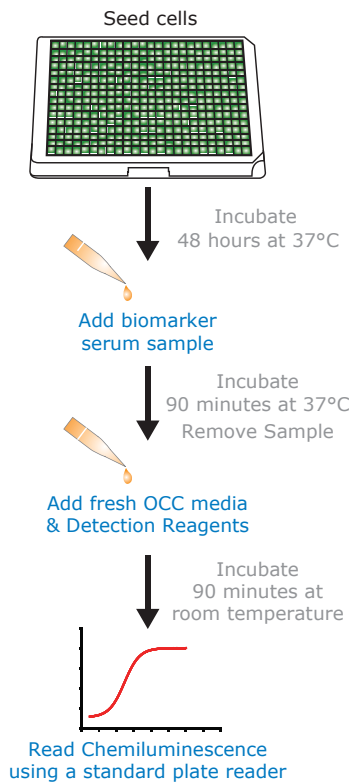


Figure 2. Simple mix-and-read PathHunter® eXpress β -Arrestin Assays can be performed in less than 3 hours

Robust and Reliable Platform for Screening Functional Biomarkers: Tolerance to High Levels of Serum and Solvents

As shown by the remarkable tolerance to high concentrations of serum and common solvents, PathHunter® eXpress β -Arrestin GPCR assays provide a highly robust, sensitive and standardized method to determine the fate of biologically active protein biomarkers in a variety of complex biological samples, such as serum, plasma, blood, ascites, and cell lysates. The sensitivity of the β -Arrestin assay was not significantly altered in media containing up to 80% serum (Fig. 3A) or in samples containing concentrations of up to 5% of common solvents (i.e., DMSO, ethanol, or methanol) (Fig. 3B). Thus, the β -Arrestin GPCR assays are highly tolerant to a wide range of serum and solvent concentrations and result in superior performance, right out of the box.

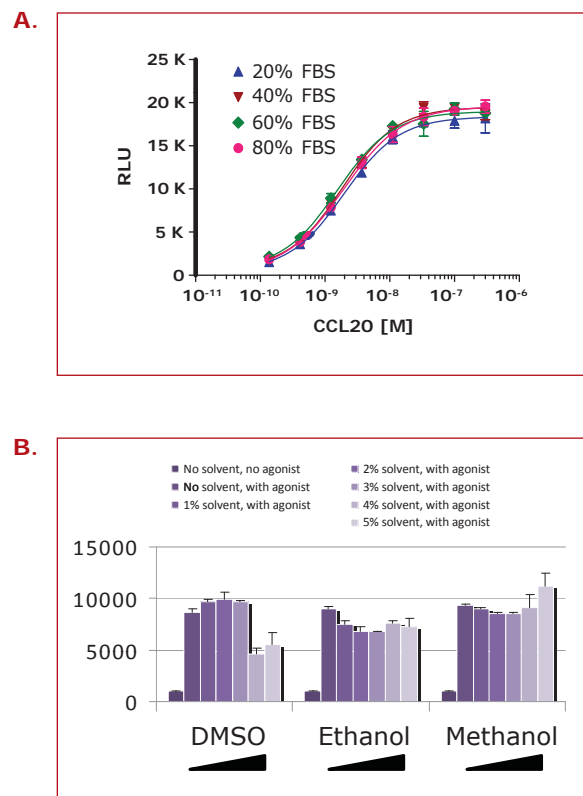


Figure 3. Use of PathHunter® eXpress assays to detect (A) ligand binding in the presence of high serum concentrations and (B) agonist in the presence of common solvents. (A) CHO-K1 cells expressing the Chemokine CCR6 receptor (Cat. # 93-0194E2C1) were seeded in 100 μ L of OCC medium. After 24 hours, medium was aspirated and replaced with fresh medium containing 0-80% FBS. Cells were incubated with varying doses of control agonist (CCL20, Cat. # 92-1005) for 90 minutes at 37°C. After the incubation, medium was aspirated and replaced with fresh OCC medium and chemiluminescent PathHunter® Detection Reagents. Plates were read after 90 minutes at room temperature. (B) CHO-K1 cells expressing the ADRB2 receptor (Cat. # 93-0182E2C1) were plated in OCC medium and incubated in the presence or absence of isoproterenol in the presence of increasing concentrations of common solvents. After the incubation, medium was aspirated and replaced with fresh PathHunter® Detection Reagents. Plates were read after 90 minutes at room temperature.

Powerful eXpress Assays Quantitatively Identify Functional Biomarkers in Patient Plasma Samples

The chemokine C-C motif ligand 5 (CCL5), which binds to the CCR5 and CCR1 chemokine receptors expressed on the surface of various types of cells (e.g., monocytes/macrophages and T lymphocytes), has been associated with atherosclerosis [1-4] and resultant cardiovascular diseases such as refractory unstable angina pectoris [5]. A series of studies using human plasma samples from a cohort of 83 patients undergoing coronary artery bypass grafting (CABG) was performed to confirm CCL5 as a biomarker for atherosclerosis. First, a standard curve of CCL5 was prepared in neat, heparinized plasma or tissue culture media and added directly to the cells (i.e., no further dilution; 100% plasma or tissue culture media in the wells). The sensitivity of the assay was not significantly altered, as shown by the remarkable similarity of the overlapping dose curves of CCL5 in plasma versus tissue culture medium (Fig. 4A). Next, functional activity of CCL5 ligand in the plasma samples from patients was assessed using the PathHunter eXpress β -Arrestin CCR5 GPCR assay. According to Spearman (non-parametric) statistical analysis of the data, the functional activity of CCL5 in the clinical samples of plasma, as determined by PathHunter eXpress β -Arrestin assays, correlated with the concentration of the chemokine in biological samples, as determined by standard immunoassay (Fig. 4B). Thus, PathHunter β -Arrestin assays are ideal tools for quantification of biologically active peptides and peptide fragments in complex biological samples, including human plasma.

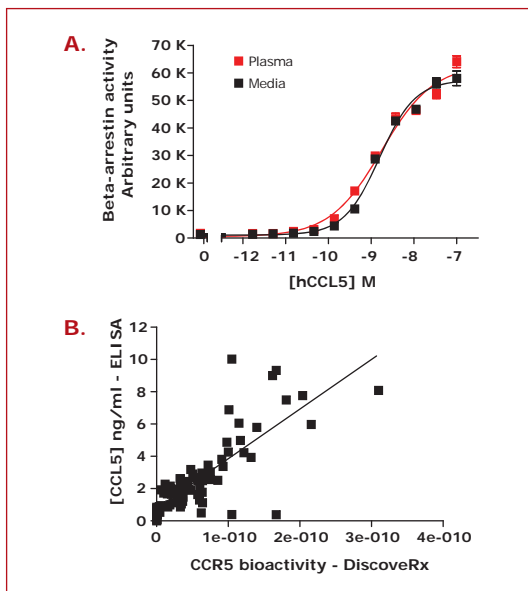


Figure 4. Use of PathHunter® eXpress β -Arrestin assays to detect biomarkers in human plasma samples. (A) Samples of either heparinized, undiluted (neat) human plasma containing human CCL5, or Ham's F12 medium containing human CCL5 were added directly to wells of CHO-K1 PathHunter eXpress CCR5 cells (Cat. #93-0224E2C1). After stimulation, plasma or Ham's F12 medium was removed, and PathHunter Detection Reagents were added and plates were read after 90 minutes at room temperature. (B) Plasma samples from 83 patients undergoing coronary artery bypass grafting (CABG) were analyzed for concentration of CCL5 by ELISA and for functional activity of CCL5 via β -Arrestin assays on CHO-K1 PathHunter eXpress CCR5 cells. Correlation coefficient = 0.78; $r^2 = 0.64$; $p = 0.0001$.

Choose From Menu of Over 250 PathHunter® eXpress β -Arrestin Kits

DiscoverX's award-winning, innovative PathHunter eXpress β -Arrestin assays can meet the rigorous demands of screening complex biological samples for desired function of biomarkers. With their unparalleled sensitivity, specificity, and ease of use, PathHunter eXpress β -Arrestin assays provide the best *in vitro* quantitative estimate of biomarkers affecting activity of known and orphan GPCRs.

DiscoverX has the broadest portfolio consisting of more than 250 human, mouse, and rat GPCR targets for discovery and analysis of biomarkers. Scaleable PathHunter eXpress β -Arrestin assays are available as ready-to-use kits, or frozen cell lines and bulk reagents.



Call toll-free **1.866.448.4864** (in the U.S.) to place your order today! For an updated list of available targets, visit www.discoverx.com/gpcrs/express_arrestin_targets.php

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