DiscoveRx
Simple Solutions for Complex Biology
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June, 2010 | Volume 4 | Issue 2

PRODUCT & SERVICES


GPCRs


Kinases


NHRs


Proteases


Screening & Profiling Services


Pathway Assays

NEWEST RELEASES

Over 60 Different assays for Human, Mouse, Canine and Rat GPCRs as well as cell-based assays for Receptor Tyrosine Kinase Targets

SPECIAL PROMOTIONS

HitHunter™ cAMP Assay Kit Today!

LumiLITE™ Microplate Reader PathHunter™ eXpress Bundle Pack
Purchase the LumiLITE™ Microplate Reader (75-0001) and *receive any 5 PathHunter eXpress kits* for a combined price of $9,995.
Promotion Code: **DRX1110**.
Offer expires 8/31/10.

DiscoveRx Assay Newsletter

Welcome to our second edition of 2010 DiscoveRx Assay Newsletter!

Once again, for your convenience, this issue is packed with information on our new product launches, novel applications, innovative assays that expedite your therapeutic programs and of course our exciting promotions! DiscoveRx continues to be a leading provider of innovative product and service solution for GPCR, Kinases and NHR. Find out how our simple assay solutions can help you with complex biological problems!

Table of Contents

What's New?

Introducing Novel cell-based PathHunter™ assay for Cytosolic Kinases — Page 3

Over 40 PathHunter™ Activated GPCR Internalization Assays Now Available — Page 4

New Targets — Page 7

Novel Applications

Discover Biased Ligands using DiscoveRx's Portfolio of Functional GPCR Screening Platforms: PathHunter™ Activated GPCR Internalization Assays, PathHunter™ β-Arrestin Recruitment & HitHunter™ cAMP Assays — Page 8

Easy Identification of G_i Antagonists and Allosteric modulators with HitHunter™ cAMP Assays — Page 10

Profile compounds against a Toxicity Panel using PathHunter NHR pro and NHR Trans assays — Page 12

Therapeutic focus

Chemokine CCR5 Receptor and Atherosclerosis — Page 14

PathHunter™ Mitotic Index Assays Biosensor Cells for Cancer Drug Discovery — Page 16

Promotions & Events!

Special Offers — Page 19

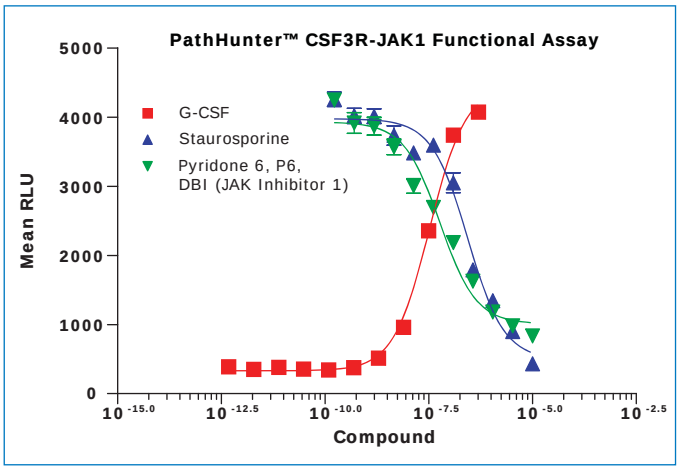
Introducing Novel cell-based PathHunter™ assay for Cytosolic Kinases

Finally a non-ELISA, screen friendly cell-based assay platform for cytokine receptors and the cytosolic tyrosine kinases.

- Saves you months of assay development
  - Simple, no wash, one-step and ready-to-screen assays
- Uses Full length receptors and kinases
  - Screen for small molecule agonists, inhibitors or functional antibodies
  - Identify biologically relevant and specific inhibitors
- One step, Direct Assays without need of Antibodies
  - Ideal for monitoring JAK1, JAK2 or JAK3 activity on cytokine receptor phosphorylation

JAK1, JAK2 and cytokine receptors PRLR, CSF3R... Read more»(http://www.discoverx.com/kinases/cell_based_ctk.php)

PathHunter™ CSF3R-JAK1 Functional Assays



	G-CSF	Staurosporine	JAK Inhibitor 1
EC_{50}	3.3051e-008	2.7882e-007	5.9411e-008

Figure 1. PathHunter™ cells expressing were incubated with increasing concentrations of the respective agonist/antagonists. PathHunter Detection Reagents (93-0001) were used in this assay. Assays were run in a 384-well plate and read on a standard luminometer. The assay detects CSF3R activation (G-CSF stimulation), CSF3R- JAK1 interaction and JAK1 inhibitors.

PathHunter™ PRLR-JAK1 Functional Assay

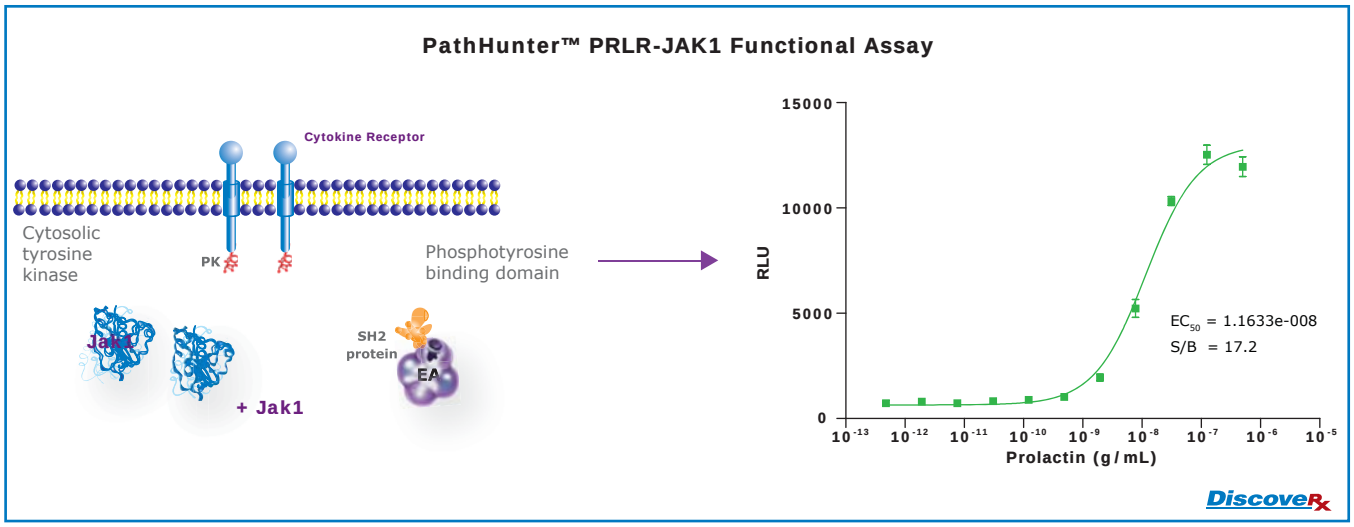
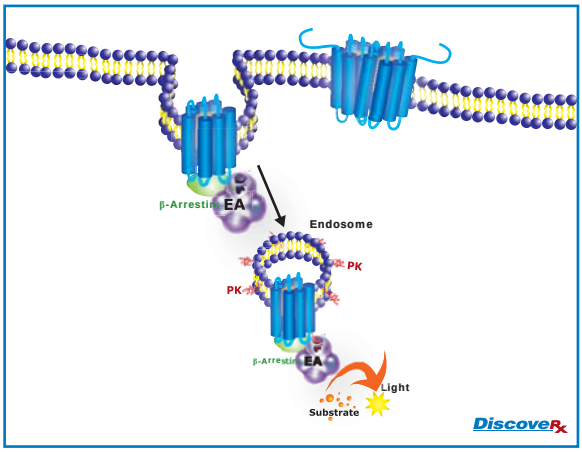


Figure 2. Agonist Dose response: Cytokine receptors lack kinase domain. They signal through the JAK/STAT pathway. PathHunter™ PRLR-JAK1 cells express Prolactin receptor and JAK1 kinase with PLCG1 as the SH2 containing domain. Upon ligand (Prolactin) binding, the PRLR gets phosphorylated. Phosphorylated cytokine receptors recruits the SH2 containing domains and this interaction forces complementation of the β-gal fragment and addition of a chemiluminescent substrate yields a signal that can be detected in any standard luminometer.

Over 40 PathHunter™ Activated GPCR Internalization Assays Now Available



- Native, untagged GPCR receptors
- Arrestin-dependent signaling pathway ideal for hit confirmation or tertiary screens
- Gain of Signal assay that detects activated GPCRs in early endosomes
- Simple, single addition protocol
- Low background and large assay windows
- No fixing, imaging or antibody labeling

PathHunter™ Activated GPCR Internalization Assays

PathHunter™ Activated GPCR Internalization assays (http://www.discoverx.com/gpcrs/endocytosis.php) provide a quantitative measurement of internalized GPCR protein localized to the early endocytic compartment. Unlike other imaging or antibody-based internalization technologies, PathHunter assays are simple, non-radioactive chemiluminescent assays that are amenable to highthroughput screening.

- Differentiate between slow and fast recyclers
- Compare pharmacology of selective and non-selective agonists
- Combine with traditional second messenger assays to identify functional antagonists, novel inhibitors or biased ligands

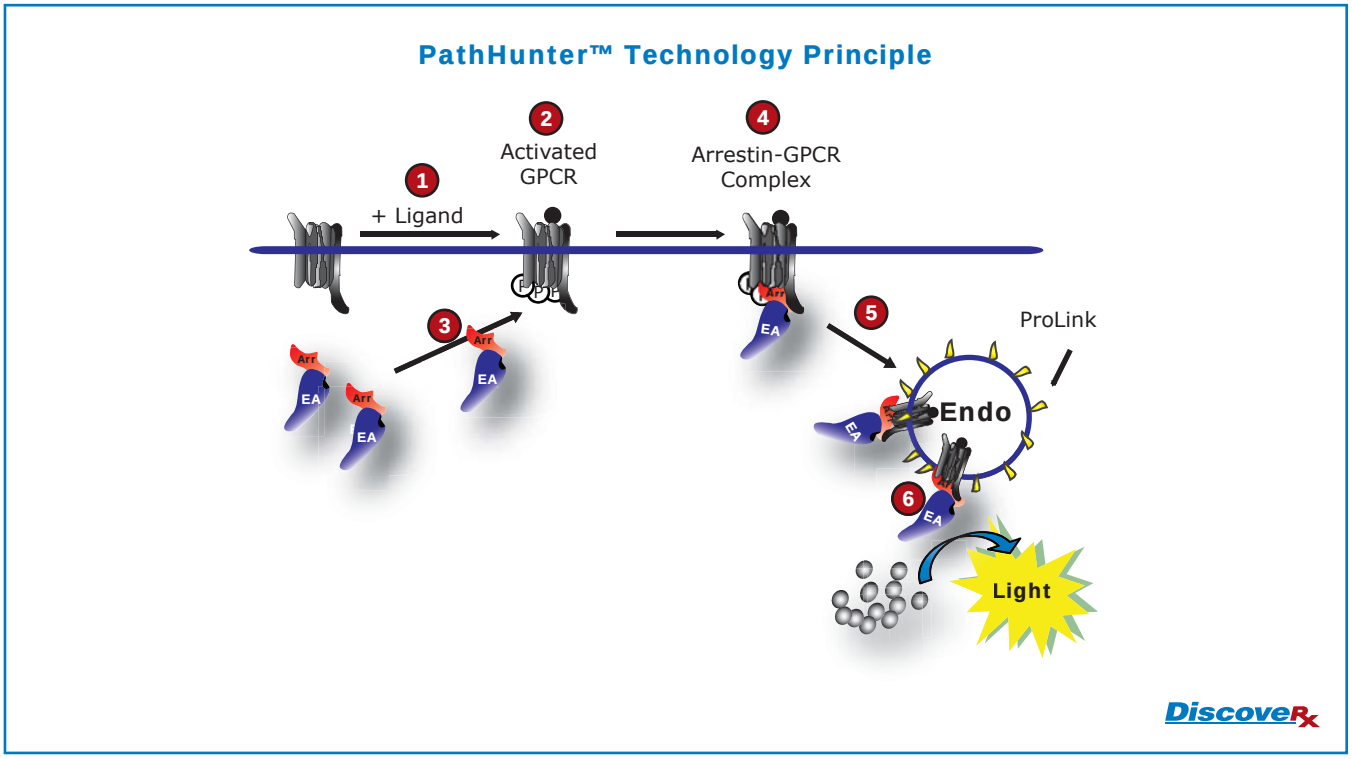


Figure 3. The small enzyme fragment of β-galactosidase (ProLink™) is localized to the surface of cellular endosomes and the larger, complementing enzyme fragment (termed Enzyme Acceptor or EA) is fused to β-Arrestin. Stimulation of the receptor results in Arrestin binding to the activated GPCR, internalization of the receptor and trafficking to cellular endosomes resulting in enzyme complementation and an increase in activity that is easily measured using PathHunter Detection Reagents.

Compound Selectivity and Specificity

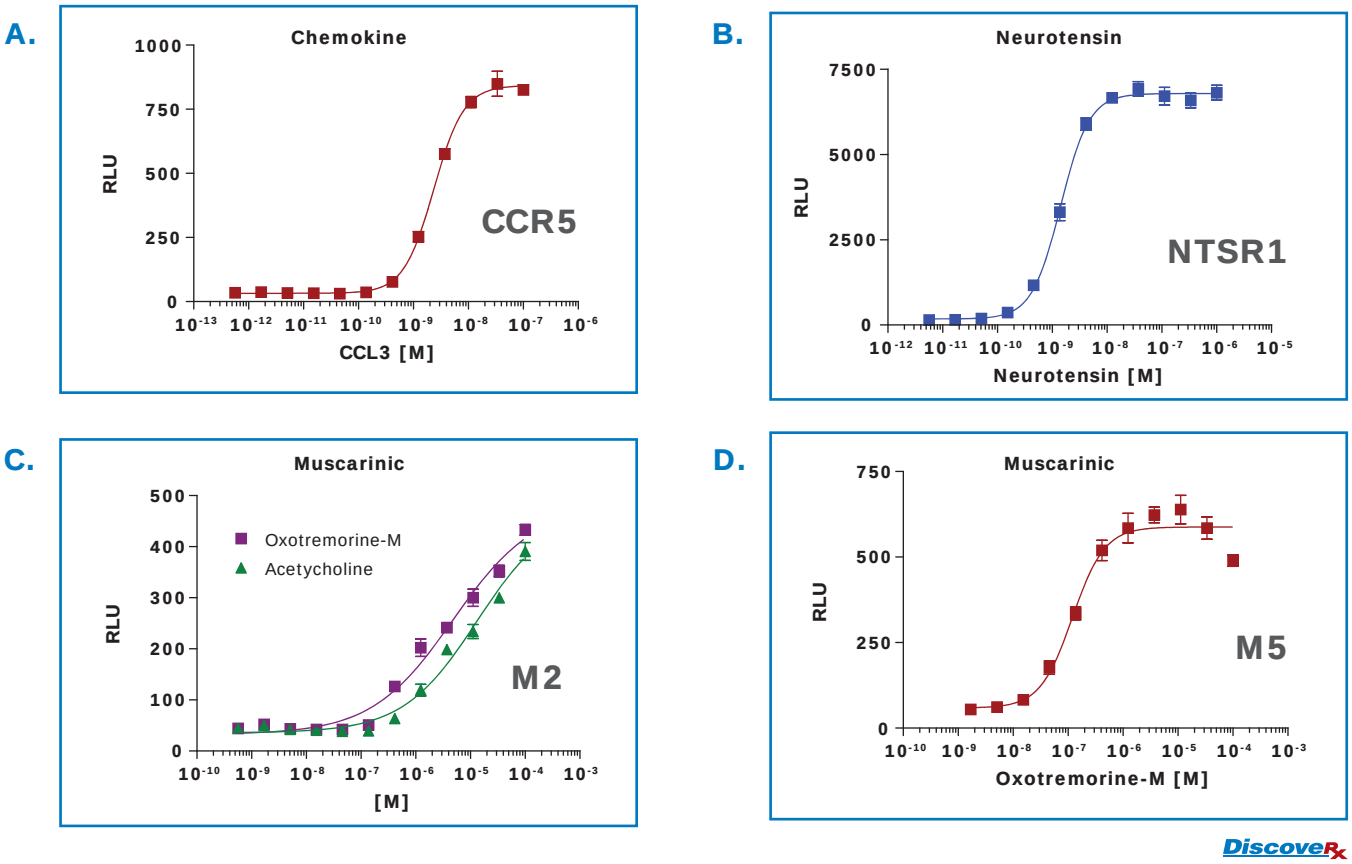


Figure 4. **(C)** Cells expressing the native, untagged Cholinergic Muscarinic M2 Receptor (CHRM2) were treated with increasing concentrations of Oxotremorine-M and Acetylcholine, two known agonists. **(A, B & D)** Agonist dose response curves wer also performed on cells expressing the Neurotensin NTSR1 **(B)**, Chemokine CCR5 **(A)** and Cholinergic Muscarinic CHRM5 **(D)** receptors. U2OS cells expressing each receptor were treated with increasing concentrations of control agonist and assayed using the PathHunter Detection Reagents. Together, this data demonstrates that the PathHunter Activated GPCR Internalization Technology is broadly applicable across a large number of GPCR families.

New Targets

New cAMP Hunter™ Gi, Gs and Gq Cell Lines
Human: BRS3, EDG2, FFAR1, FFAR3, PTGER1, RXFP4, SSTR5, 5-HT1A, OPRM1

New PathHunter™ Receptor Tyrosine Kinase Assays
Human: ErbB1, DDR1, DDR2

New PathHunter™ Cytokine Receptors, cytosolic tyrosine kinase assays
Human: CSF3R-JAK1, PRLR-JAK1, PRLR-JAK2

New PathHunter Pathway Assays
Human: FOXO3 translocation, IκB degradation

New PathHunter™ β-Arrestin Assays
Human: BRS3, DRD4, EDG6, 5HT6, HTR1A, HTR1B, HTR1E, HTR1F, HTR5A, LHCGR, MCHR2, RXFP3, RXFP4
Mouse: ADORA3, CCR6, CX3CR1, CXCR7 (CMKOR1), EDG6, EDNRA, VIPR1

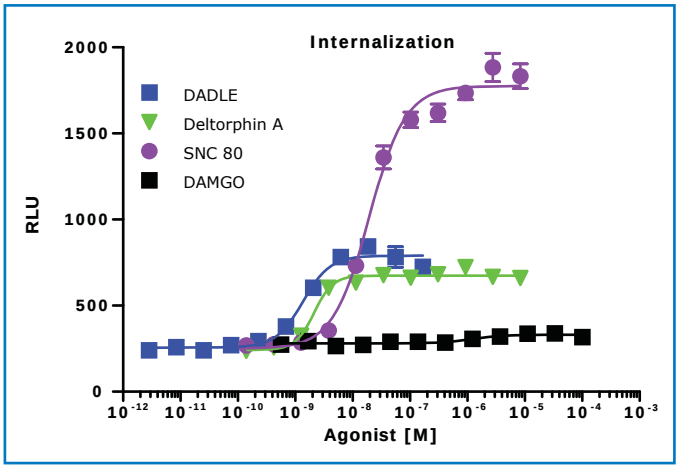
New PathHunter™ Orphan Assays
Human: ADMR (GPR182), GPR6, GPR20, GPR31, GPR50, GPR85, TAAR5

New PathHunter™ Activated GPCR Internalization Assays
Human: AGTRL1, AVPR1B, AVPR2, BDKRB2, C5R1, CCR4, CCKBR, CMKLR1, CRHR1, HCRTR2, NPY2R, OPRD1, OXTR1, P2RY11, TACR1, VIPR2

New PathHunter™ β-Arrestin eXpress Kits
Human: AVPR2
Mouse: C5AR1
Rat: CRTH2

New EasyScreen™ β-Arrestin Kits
Human: AVPR2, MC1R
Mouse: C5R1
Rat: CRTH2

Discover Biased Ligands using DiscoveRx's Portfolio of Functional GPCR Screening Platforms: PathHunter™ Activated GPCR Internalization Assays, PathHunter™ β-Arrestin Recruitment & HitHunter™ cAMP Assays



Don't miss out on new information about your lead compound!

- Over 400 cellular, whole cell GPCR assays available for second messenger signaling, arrestin recruitment and receptor internalization
- Combine with traditional second messenger assays to uncover compound-specific receptor recycling profiles

Uncovering Biased Ligands with DiscoveRx's Comprehensive GPCR Assay Portfolio

The δ-opioid receptor (OPRD1) controls the body's response to pain. Activation of OPRD1 alleviates persistent pain while prolonged agonist stimulation can lead to reduced responsiveness, or receptor desensitization and depending on the ligand, correlates with rapid receptor internalization. When combined with standard second messenger and PathHunter™ β-Arrestin recruitment assays, PathHunter™ Activated GPCR Internalization (http://www.discoverx.com/gpcrs/endocytosis.php) assays can provide a rapid and efficient method for determining compound-specific differences in receptor activation and internalization.

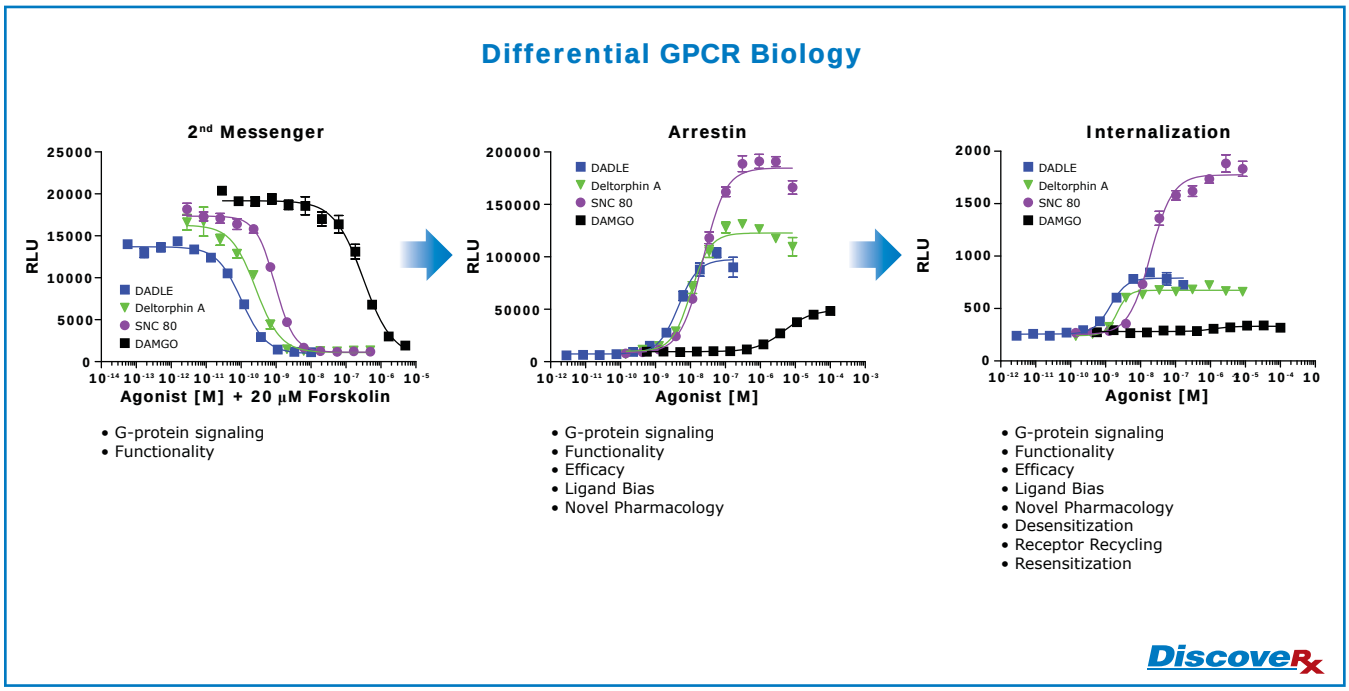
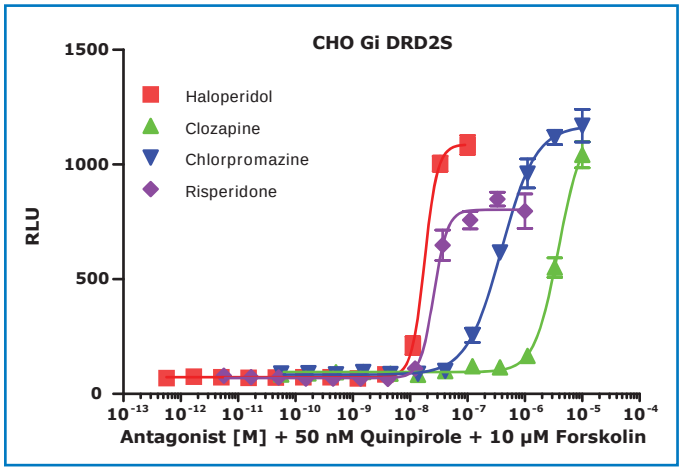


Figure 1. cAMP Hunter™, PathHunter™ Arrestin and PathHunter™ Activated GPCR Internalization cell lines expressing the human OPRD1(d) receptor were treated with increasing concentrations of known agonists and assayed using the PathHunter Detection Reagents. In addition to information on G-protein signaling, PathHunter Activated GPCR Internalization assays provide a non-imaging, non-radioactive chemiluminescent approach for determining novel pharmacology of lead compounds and receptorre-cycling and or desensitization/resensitization kinetics.

Easy Identification of Gi Antagonists and Allosteric modulators with HitHunter™ cAMP Assays



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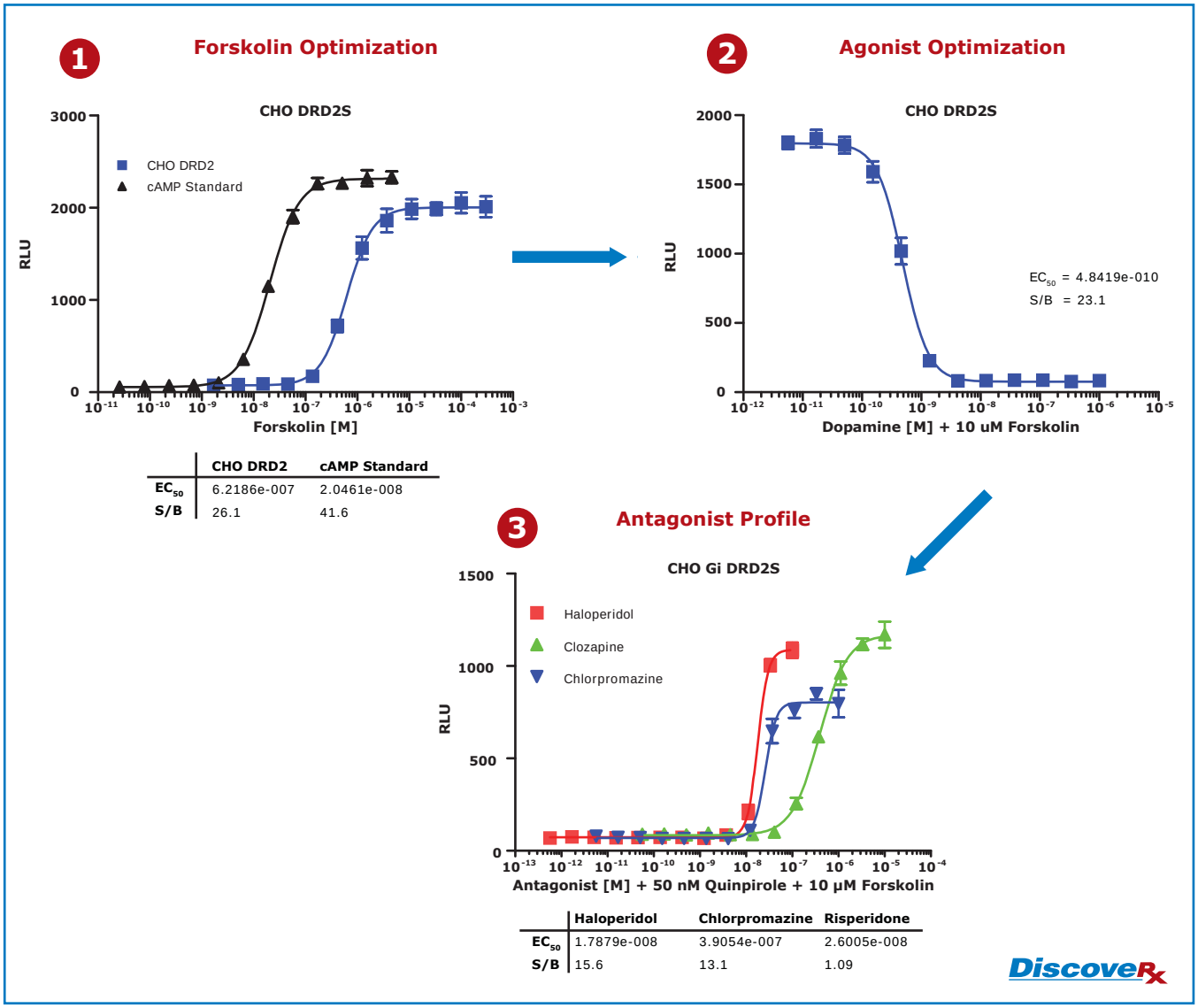
	Haloperidol	Clozapine	Chlorpromazine	Risperidone
EC_{50}	1.7879e-008	3.8828e-006	3.9054e-007	2.6005e-008

Identifying Gi Antagonists and/or allosteric modulators using cAMP response requires an assay platform that offers large assay windows and positive gain of signal response. With HitHunter cAMP, you are assured of

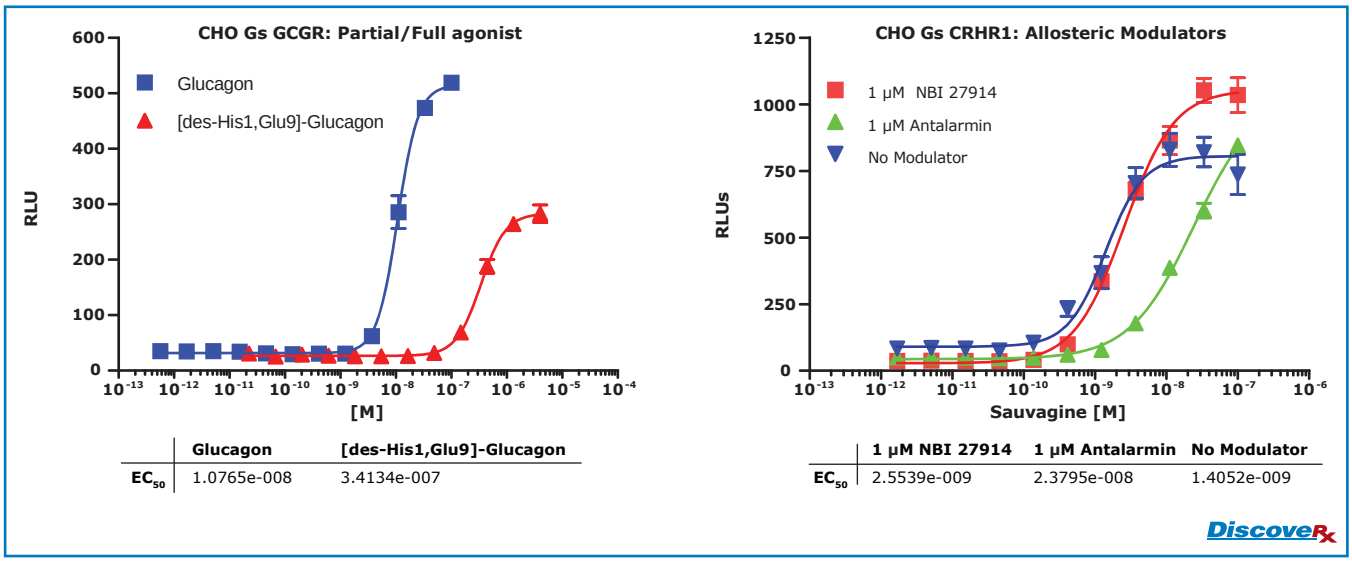
- Large Assay windows (> 45 in standard curve)
- Gain of signal assay format that simplifies detection of weak antagonists
- Chemiluminescent detection that reduces false positives
- Sensitivity and robustness allow for easy identification of positive or negative allosteric modulators or partial agonists



	CHO DRD2	cAMP Standard
EC_{50}	6.2186e-007	2.0461e-008
S/B	26.1	41.6

	Haloperidol	Chlorpromazine	Risperidone
EC_{50}	1.7879e-008	3.9054e-007	2.6005e-008
S/B	15.6	13.1	1.09

Figure 2. cAMP response was measured in cAMP Hunter Dopamine receptor D2 (short variant) using HitHunter cAMP XS+ assay. One of the distinguishing feature of the HitHunter kits are large assay windows. Starting with a Large assay window in forskolin optimization helped in retaining the window when profiling Gi antagonists.

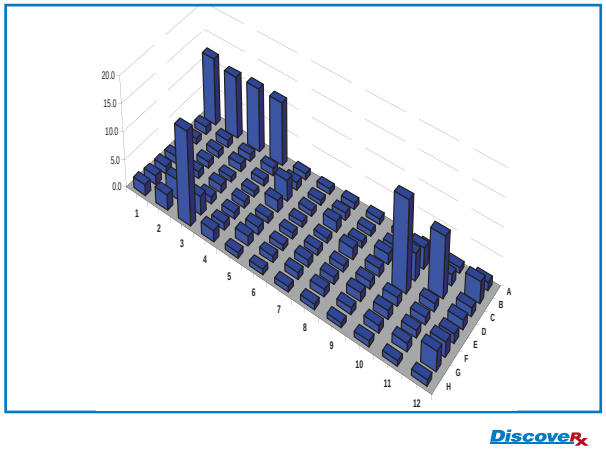


	Glucagon	[des-His1,Glu9]-Glucagon
EC_{50}	1.0765e-008	3.4134e-007

	1 µM NBI 27914	1 µM Antalarmin	No Modulator
EC_{50}	2.5539e-009	2.3795e-008	1.4052e-009

Figure 3. cAMP response was measured in cAMP Hunter Glucagon receptor (GCGR) and Corticotrophin receptor (CRHR1) using HitHunter cAMP XS+ assay. HitHunter cAMP assays are gain of signal assays that make it easier to detect partial agonists. Sensitivity and large assay windows make HitHunter cAMP assays ideal for allosteric compounds.

Profile compounds against a Toxicity Panel using PathHunter NHR_{PRO} and NHR_{TRANS} assays



A novel, non-reporter gene assay for measuring NHR translocation and co-activator interaction

- Panel includes PPARgamma, LXR, FXR and others
- 3 hour direct assay make screening and profiling of compounds easier than ever
- Want to test our panel, send in your compounds to our profiling group!

DiscoveRx has the most comprehensive portfolio of Cell-Based Nuclear Hormone Receptors Assays. Read more» (http://www.discoverx.com/nhrs/prod-nhrs.php)

Study Agonists, inverse agonists and antagonists with PathHunter™ NHR assays

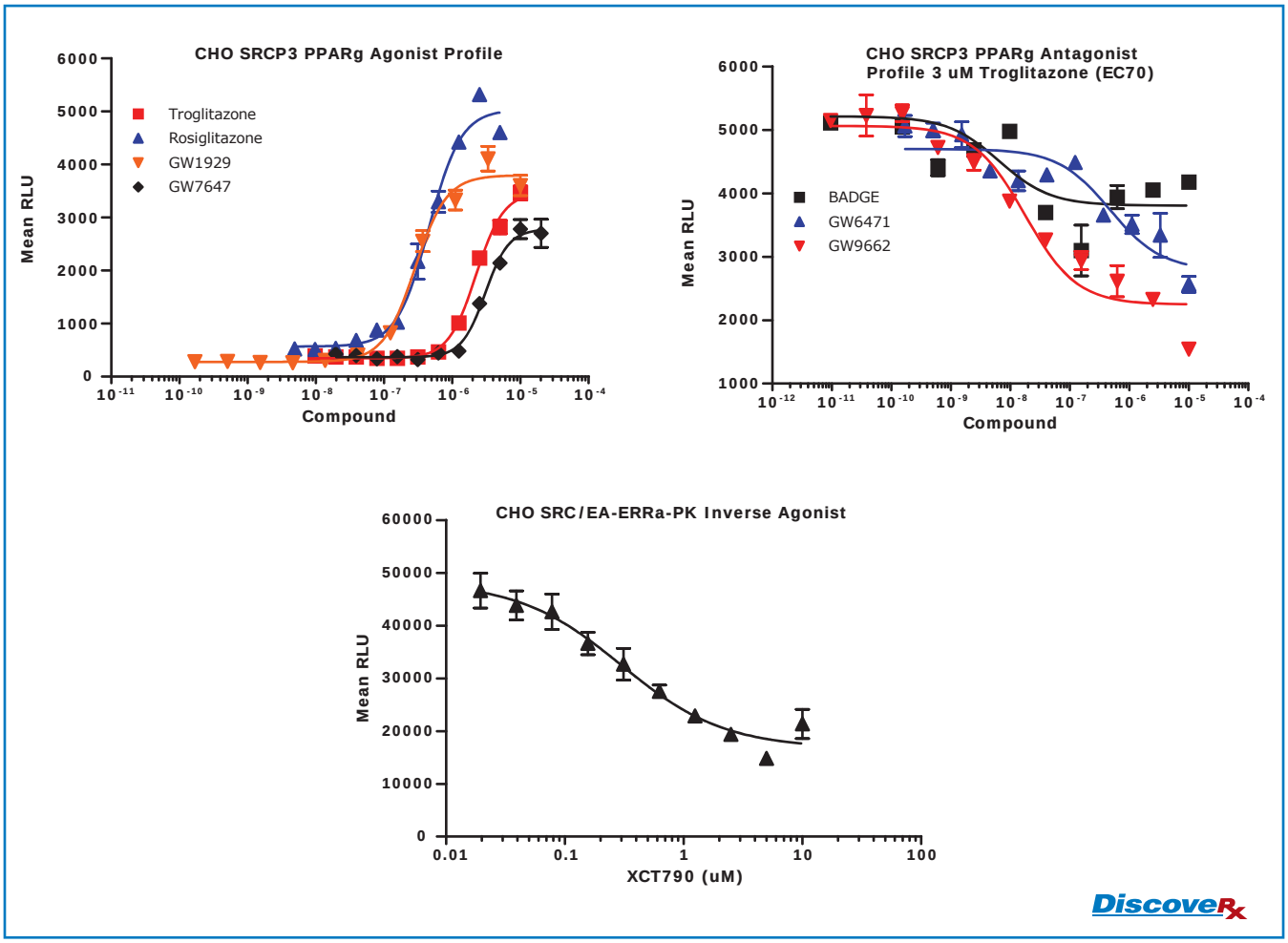
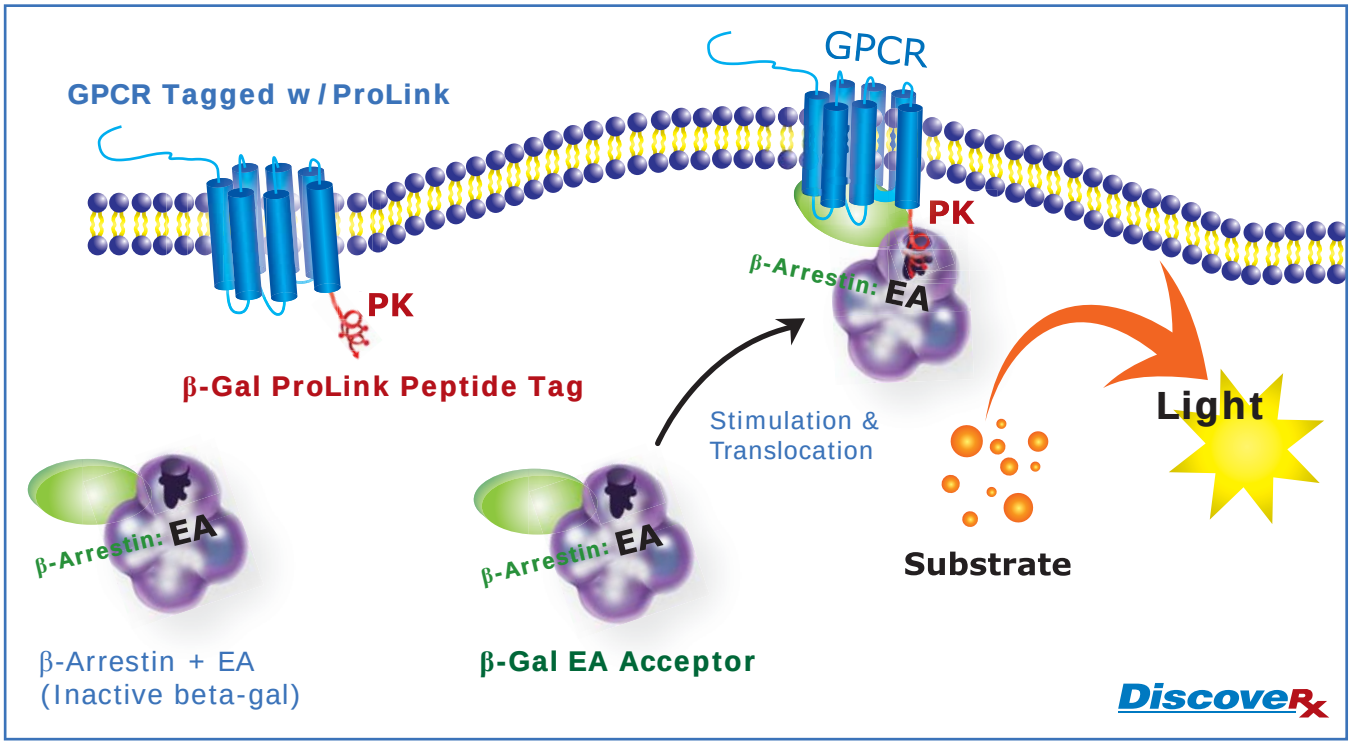


Figure 4. PathHunter NHR assay is a one-step cell-based assay ideal for screening or profiling of agonists, antagonists or inverse agonists. The above data shows proof data on 2 receptors: PPARγ and ERRα.

Chemokine CCR5 Receptor and Atherosclerosis

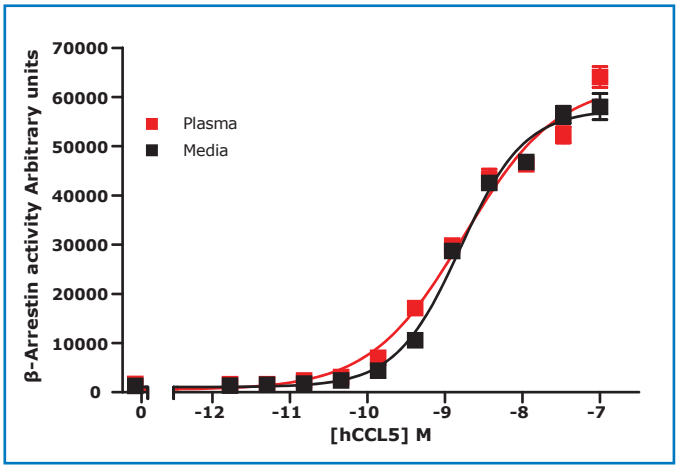


Atherosclerosis affects 64 million Americans and is the condition in which an artery wall thickens as the result of a build-up of fatty materials. The proatherogenic chemokine ligand, CCL5, binds to the CCR1 and CCR5 chemokine receptors expressed on various cell types involved in atherosclerotic plaque formation and is therefore considered prognostic for disease progression. Read more from Zernecke et al in their review on Chemokines in Atherosclerosis.

Many chemokines undergo proteolytic processing which can generate cleaved products that are either more active, antagonistic or inactive compared to the native form. While CCL5 can be detected by standard immunoassays (ELISA), an assay that measures chemokine bioactivity direct in plasma samples may be more useful than a standard ELISA.

DiscoveRx offers the CCR5 receptor in both the assay-ready and live cell formats.

- View assay-ready performance using neat heparinized plasma
- View assay-ready vs ELISA performance for a cohort of coronary bypass patients



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** Data generated by Gemma White and kindly provided by Dr. David* Greaves of Oxford University

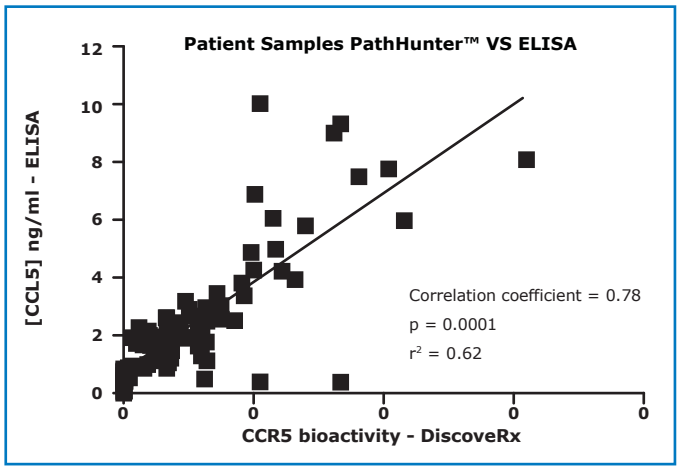

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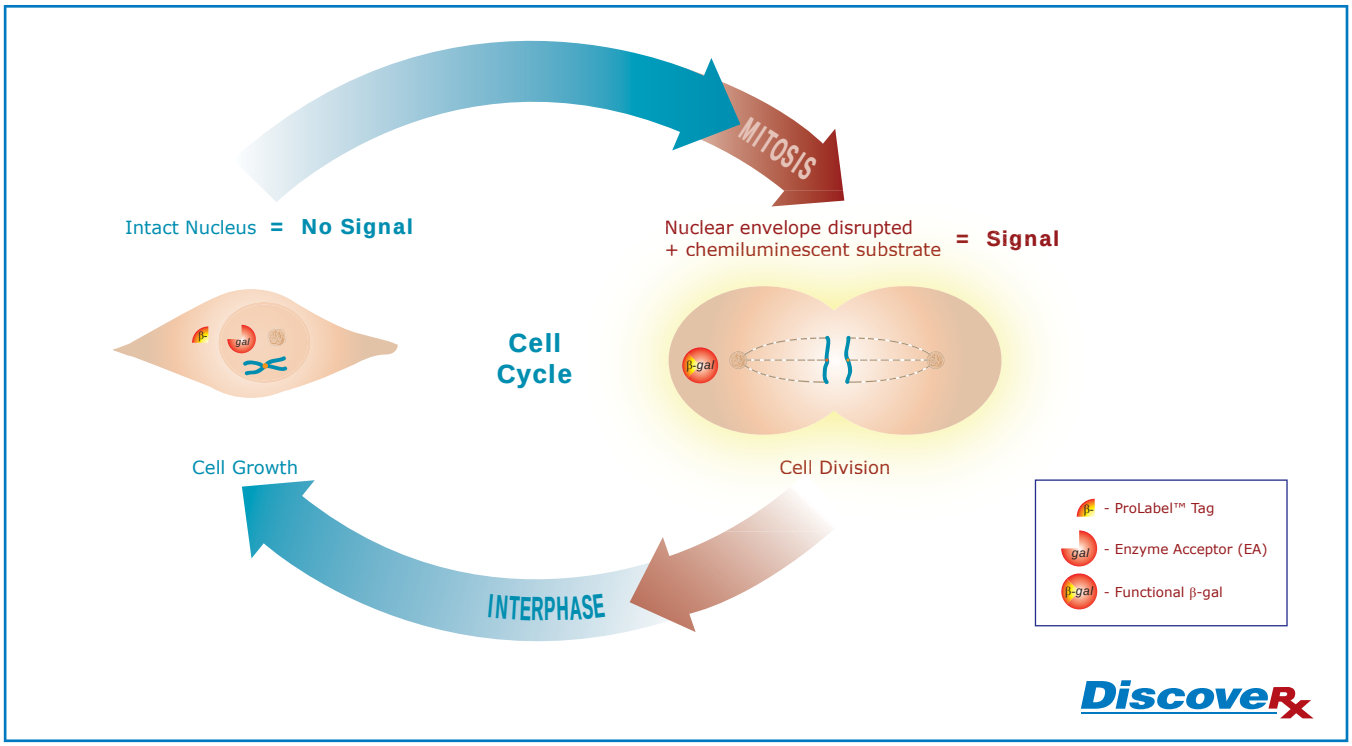
Figure 1. Plasma samples from 83 coronary artery bypass grafting (CABG) patients were tested in the PathHunter eXpress CCR5 β-Arrestin GPCR Assay and results werecompared in a standard ELISA format.

** Data generated by Gemma White and kindly provided by Dr. David* Greaves of Oxford University

PathHunter™ Mitotic Index Assays



Biosensor Cells for Cancer Drug Discovery

Cancer occurs as a result of altered regulation of cell cycle leading to unchecked cell proliferation and subsequent tumor formation. Expectedly, most of the cancer therapeutics target cell cycle and cell proliferation events. The PathHunter™ Mitotic Index Assays are cell-based assays specifically designed to identify compounds that affect the various stages of the mammalian cell cycle and are therefore perfectly suited to be used in Cancer Drug Discovery. These cell lines are engineered with nuclear (EA) - and cytoplasmic-localized (PL) fragments of β-galactosidase (β-gal). At mitosis, the nuclear envelope breaks down, allowing the two β-gal fragments (EA and ProLabel™) to complement forming an active enzyme which subsequently hydrolyzes a chemiluminescent substrate to generate signal.

- Offer HEK 293, CHO-K1 and U2OS double-stable cell lines
- Homogeneous (no wash steps), cell-based assays
- Identify compounds affecting various stages of the cell cycle
- Identify siRNAs or biologics that inhibit proliferation in mammalian cells

For additional cancer-related assays offered by DiscoveRx, please visit:
(http://www.discoverx.com/charts/cancer_gpcr_chart.php)
(http://www.discoverx.com/charts/cancer_kinase_chart.php)
(http://www.discoverx.com/charts/cancer_nhr_protease_chart.php)

Differentiate Microtubule from DNA Replication Inhibitors

The assays are sensitive and specific enough to differentiate between inhibitors of mitosis and other cell cycle phases, such as DNA replication in S phase.

Key Assay Features

- Whole cell, functional assay
- Completely homogenous (no wash steps or media exchange)
- Fully compatible with automated HTS
- No fixation, no imaging, no antibody labeling

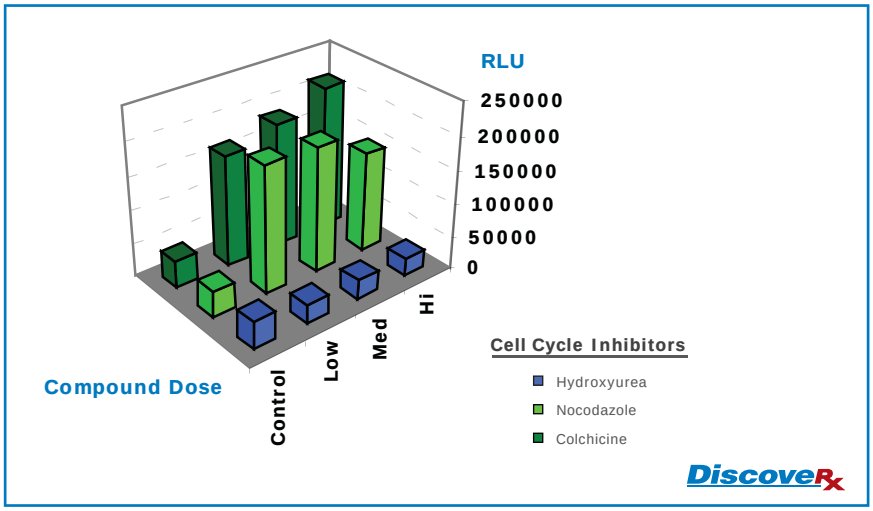


Figure 2. PathHunter™ CHO-K1 Mitotic Index cells were treated with three compounds known to interfere with cell cycle at different phases. Two microtubule inhibitors (Nocodazole and Colchicine) increased assay signal, indicating that cells have been arrested at the nuclear envelope breakdown stage, hence allowing for complementation to occur. Hydroxyurea, a known DNA replication inhibitor, did not cause an increase in signal as it arrests the cell cycle in S phase, thereby maintaining an intact nuclear envelope, hence no complementation.

Identification of Cancer Targets with siRNA

The assays can be used for siRNA studies, as biosensors to identify novel cancer targets.

Key Assay Features

- Achieve high transfection efficiency with siRNA
- Extremely sensitive and very quantitative
- Ideal for screening siRNAs against novel mitotic genes
- Fully compatible with automated HTS
- No fixation, no imaging, no antibody labeling

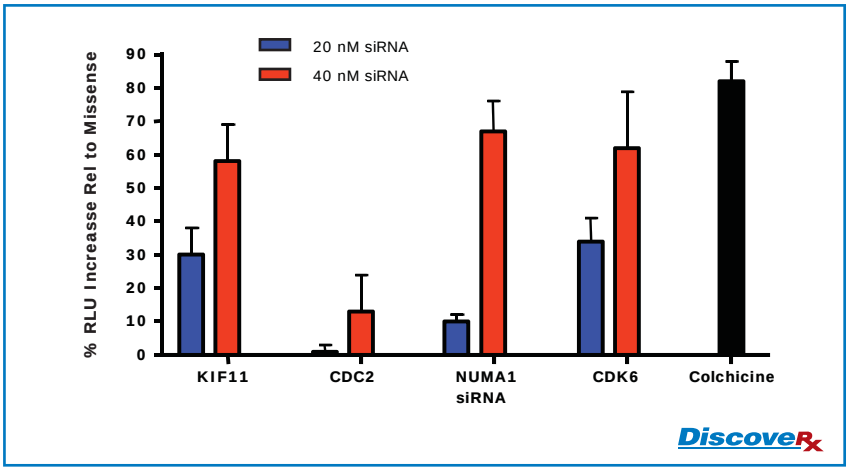


Figure 3. PathHunter™ CHO-K1 Mitotic Index cells were transfected with varying concentrations of siRNAs targeting mitotic genes. siRNA-mediated knockdown of these mitotic genes resulted in an increase in signal, indicative of mitotic arrest. Colchicine was used as a positive control.

Promotions & Events!

HitHunter™ cAMP Assay Kit Today!

Try or request an **on-site demo** with any ***HitHunter™ cAMP Assay Kit for FREE!*** Click here (http://204.200.218.3/DRx_HHcAMP_fsample_landing_0610.html) for more information. *Offer expires August 31, 2010. Offer available for only to first time HitHunter™ cAMP kit buyers. Limit 1 HitHunter™ cAMP kit per customer.*

LumiLITE™ Microplate Reader & PathHunter™ eXpress Bundle Pack

Get started today with DiscoveRx technology! ***Purchase the LumiLITE™ Microplate Reader (75-0001) and receive any 5 PathHunter eXpress kits*** for a combined price of $9,995. Promotion Code: **DRX1110**. *Offer expires 8/31/10.*

PathHunter™ Internalization Cell Lines

Get a FREE Demo and ***FAS Service*** on any PathHunter™ Activated Internalization Assay. Promotion Code: **DRX1010**. *Offer expires 8/31/10.*

PathHunter™ Activated Internalization Cell Lines

Buy any PathHunter™ Activated GPCR Internalization cell line and get ***30% off***. Promotion Code: **DRX0810**. *Offer expires 6/30/10.*

PathHunter™ β-Arrestin Human and Second Messenger Cell Lines

Buy a Human PathHunter™ β-Arrestin cell line and receive any ***Second Messenger cell line for half price***. Promotion Code: **DRX0910**. *Offer expires 8/31/10.*