

PathHunter® HEK 293 ADRA1D(82-572) β -Arrestin Cell Line

Catalog Number: 93-0983C1 **Lot Number:** See Vial
Contents: 2 vials, 1 x 10⁶ cells per vial in 1 mL

Background

PathHunter β -Arrestin GPCR cell lines are engineered to co-express the ProLink™ (PK) tagged GPCR and the Enzyme Acceptor (EA) tagged β -Arrestin. Activation of the GPCR-PK induces β -Arrestin-EA recruitment, forcing complementation of the two enzyme fragments. The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal. PathHunter cell lines expressing Gq-coupled receptors can also be used to detect calcium mobilization.

Product Information

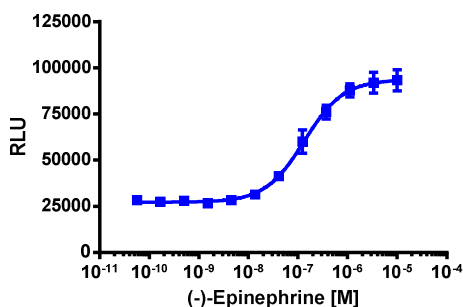
Target GPCR: ADRA1D(82-572)
Description: Adrenergic receptor alpha 1D
Receptor Family: Adrenoceptors **β -Arrestin Isoform:** β -Arrestin-2
Accession Number: NM_000678 **ProLink™ Tag:** PK1
Coupling: Gq **Cell Type:** HEK 293
GPCR Species: Human
Storage: Short term (<24 h): Store at -80°C; Long term (>24 h): Store in vapor phase of liquid nitrogen.

Functional Performance

Cells were plated in a 384-well plate and incubated overnight at 37°C and 5% CO₂ to allow the cells to attach and grow. Cells were then stimulated with a control agonist using the assay conditions described below. Following stimulation, signal from Arrestin recruitment was detected using the PathHunter Detection Kit according to the recommended protocol. Calcium mobilization was detected using the Calcium No Wash^{PLUS} detection kit. Please refer to page 2 for recommended assay reagents, detection reagents, and control compounds.

PathHunter Arrestin Assay

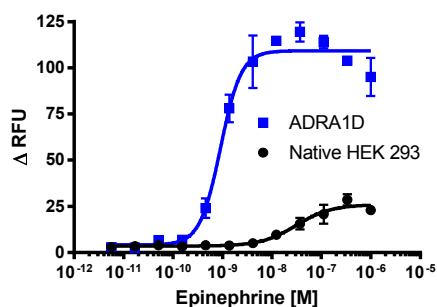
Cell Number/Well: 5000
Agonist Incubation Time (minutes): 90
Agonist Incubation Temp. (°C): 37



EC₅₀ for Arrestin Recruitment (nM): 134.6
Signal:Background at agonist E_{max}: 3.3

Ca²⁺ Mobilization Assay

Cell Number/Well: 10000
Signal Read Time (@ 2 sec intervals): 2 minutes
Signal Read Temperature (°C): RT



EC₅₀ for Calcium Mobilization (nM): 0.9

Note: This cell line was developed and quality control tested via the PathHunter Arrestin Assay only. Calcium mobilization assay was run independently from the PathHunter Arrestin Assay. Calcium data is background subtracted and represented as Δ RFU (Relative Fluorescence Units).

Important! This assay requires an additional step: Please refer to Additional Protocol Information section.

Passage Stability

This cell line has been confirmed to be stable through 10 passages with no significant drop in assay window or change in EC₅₀. Passage stability testing was conducted using the PathHunter Arrestin Assay only.

Mycoplasma Testing

This lot was tested and found to be free of mycoplasma contamination. Data available upon request.

Required Materials

The following additional materials are required but not provided:

| Product Use* | Product Description | Catalog Number |
|----------------------------|--|----------------|
| Detection | PathHunter® Detection Kit | 93-0001 |
| Ca ²⁺ Detection | Calcium No Wash ^{PLUS} | 90-0091 |
| Cell Culture | AssayComplete™ Cell Culture Kit-105 | 92-3105G |
| Cell Plating | AssayComplete™ Cell Plating 7 Reagent | 93-0563R7A |
| Cell Detachment | AssayComplete™ Cell Detachment Reagent | 92-0009 |
| Cell Thawing | AssayComplete™ Thawing Reagent T1 | 92-4101TR |
| Cell Freezing | AssayComplete™ Freezing Reagent F1 | 92-5101FR |

*Please inquire about our cell line-specific AssayComplete Starter Packs to get you started with your cell culture needs.

Required Antibiotics

| Antibiotic Name | Concentration (µg/mL) | Catalog Number |
|-----------------------------|-----------------------|----------------|
| AssayComplete™ Puromycin | Not Applicable | Not Applicable |
| AssayComplete™ Hygromycin B | 200 | 92-0029 |
| AssayComplete™ G418 | 800 | 92-0030 |

Additional Ligand Information

Control Compound: (-)-Epinephrine

Vendor: DiscoverX® (Catalog No. 92-1284)

Additional Protocol Information

Important Note: ADRA1D (NM_000678) truncated to ADRA1D(82-572)

For order placement or technical support, please call 1.866.448.4864 (North America) or +44.121.260.6142 (Europe) or e-mail info@discoverx.com. For additional information, please visit discoverx.com.

Limited Use License Agreement

These products may be covered by issued US and/or foreign patents, patent application and subject to Limited Use Label License.

Please visit discoverx.com/license for a list of products that are governed by limited use label license terms and relevant patent and trademark information.