

PathHunter® CHO-K1 CHRM5 β -Arrestin Cell Line

Catalog Number: 93-0519C2 **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

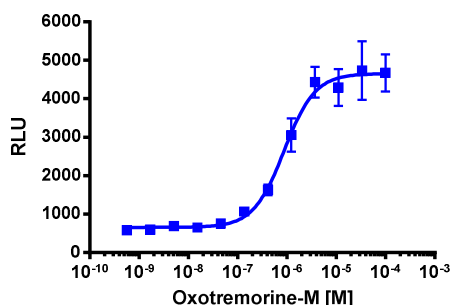
Target GPCR: CHRM5
Description: Cholinergic receptor, muscarinic 5
Receptor Family: Acetylcholine **β -Arrestin Isoform:** β -Arrestin-2
Accession Number: AF498919 **ProLink™ Tag:** ARMS2-PK2
Coupling: Gq **Cell Type:** CHO-K1
GPCR Species: Human
Storage: Short term (<24 h): Store at -80°C; Long term (>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): 879.6

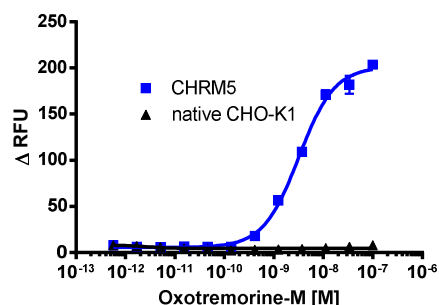
Signal:Background at agonist E_{max}: 8

Control Antagonist: Atropine Arrestin Assay IC₅₀ (nM): 1.3

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).

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): 3.2

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-107 | 92-3107G |
| Cell Plating | AssayComplete™ Cell Plating 2 Reagent | 93-0563R2A |
| Cell Detachment | AssayComplete™ Cell Detachment Reagent | 92-0009 |
| Cell Thawing | AssayComplete™ Thawing Reagent T2 | 92-4102TR |
| Cell Freezing | AssayComplete™ Freezing Reagent F2 | 92-5102FR |

*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 | 300 | 92-0029 |
| AssayComplete™ G418 | 800 | 92-0030 |

Additional Ligand Information

Control Compound: Oxotremorine-M

Vendor: DiscoverX® (Catalog No. 92-1106)

Additional Prolink™ Tag Description

PK2 is a slight variant of PK1 and has been shown to enhance EFC. ARMS (Arrestin Recruitment Modulating Sequence) is an 18-21 amino acid spacer between the GPCR and the PK tag and has been shown to enhance β-Arrestin recruitment.

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.

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