

PathHunter® U2OS CHRM3 β -Arrestin Cell Line

Catalog Number: 93-0860C3 **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

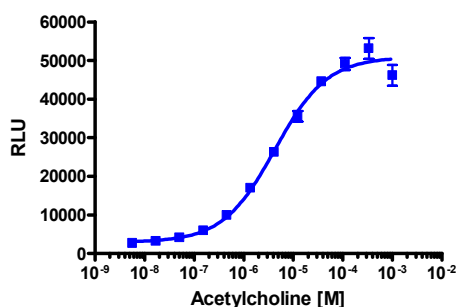
Target GPCR: CHRM3
Description: Cholinergic receptor, muscarinic 3
Receptor Family: Acetylcholine **β -Arrestin Isoform:** β -Arrestin-2
Accession Number: AF498917 **ProLink™ Tag:** PK1
Coupling: Gq **Cell Type:** U2OS
GPCR Species: Human
Storage: Short term (<24 h): Store at -80°C; Long term (>24 h): 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

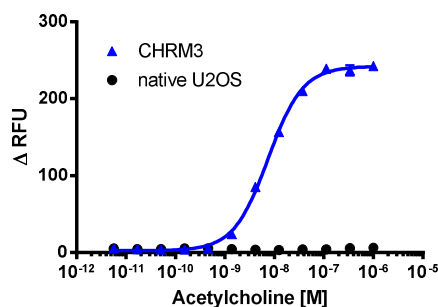
Cell Number/Well: 5000
Agonist Incubation Time (minutes): 90
Agonist Incubation Temp. (°C): 37



EC₅₀ for Arrestin Recruitment (nM): 4240
Signal:Background at agonist E_{max}: 17.2

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): 7.5

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).

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-103	92-3103G
Cell Plating	AssayComplete™ Cell Plating 5 Reagent	93-0563R5A
Cell Detachment	AssayComplete™ Cell Detachment Reagent	92-0009
Cell Thawing	AssayComplete™ Thawing Reagent T3	92-4103TR
Cell Freezing	AssayComplete™ Freezing Reagent F3	92-5103FR

*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	250	92-0029
AssayComplete™ G418	500	92-0030

Additional Ligand Information

Control Compound: Acetylcholine

Vendor: DiscoverX® (Catalog No. 92-1125)

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.