

See Vial

PathHunter[®] CHRM1 Activated GPCR Internalization U2OS Cell Line

| Catalog Number: | 93-0655C3 | Lot Number: |
|-----------------|--------------------------------------------|-------------|
| Contents: | 2 vials, 1 x 10^6 cells per vial in 1 mL | |

Background

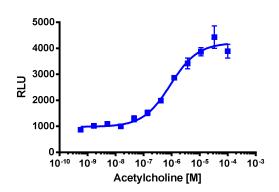
PathHunter Activated GPCR Internalization cell lines are engineered to co-express an untagged GPCR, Enzyme Acceptor (EA) tagged β -Arrestin, and a ProLinkTM (PK) tag localized to the endosomes. Activation of the GPCR induces β -Arrestin recruitment, followed by internalization of the Receptor/Arrestin-EA complex in PK-tagged endosomes. This forces complementation of the two β -galactosidase enzyme fragments (EA and PK), forming functional enzyme that hydrolyzes substrate to generate a chemiluminescent signal.

Product Information

| Target GPCR: | CHRM1 |
|-------------------------|-------------------------------------------------------------------------------------------------|
| Description: | Cholinergic receptor, muscarinic 1 |
| Receptor Family: | Acetylcholine |
| Coupling: | Gq |
| Accession Number: | AF498915 |
| GPCR Species: | Human |
| β-Arrestin Isoform: | β-Arrestin-2 |
| Cell Type: | U2OS |
| 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% CO ₂ 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 was detected using the PathHunter Detection Kit according to the recommended protocol. Please refer to page 2 for recommended assay reagents, detection reagents, and control compounds.



| Cell Number/Well: | 5000 |
|-------------------------------------------------|---------------|
| Control Agonist: | Acetylcholine |
| Agonist Incubation Time (minutes): | 180 |
| Agonist Incubation Temperature (°C): | RT |
| EC_{50} for Agonist Stimulation (nM): | 900.4 |
| Signal:Background at Agonist E _{max} : | 5.0 |

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Passage Stability

This cell line has been confirmed to be stable through 10 passages with no significant drop in assay window or change in EC_{50} .

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 |
| 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 | 0.25 | 92-0028 |
| AssayComplete™ Hygromycin B | 250 | 92-0029 |
| AssayComplete™ G418 | 500 | 92-0030 |

Additional Ligand Information

Control Agonist: 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.

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