

PathHunter® EDG5 Activated GPCR Internalization U2OS Cell Line

Catalog Number: 93-0735C3 **Lot Number:** See Vial
Contents: 2 vials, 1 x 10⁶ cells per vial in 1 mL

Background

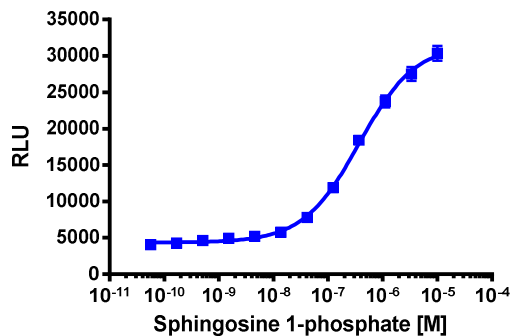
PathHunter Activated GPCR Internalization cell lines are engineered to co-express an untagged GPCR, Enzyme Acceptor (EA) tagged β -Arrestin, and a ProLink™ (PK) tag localized to the endosomes. Activation of the GPCR induces β -Arrestin recruitment, followed by internalization of the Receptor/Arrestin-EA 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: EDG5
Description: Sphingosine 1-phosphate receptor 2
Receptor Family: Lysophospholipid (S1P)
Coupling: Gs
Accession Number: NM_004230.3
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% 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 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: Sphingosine 1-phosphate
Agonist Incubation Time (minutes): 180
Agonist Incubation Temperature (°C): 37
EC₅₀ for Agonist Stimulation (nM): 370.8
Signal:Background at Agonist E_{max}: 7.5

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.

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 28 Reagent | 93-0563R28A |
| 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: Sphingosine 1-phosphate

Vendor: DiscoverX[®] (Catalog No. 92-1118)

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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