

PathHunter® EDG3 Activated GPCR Internalization U2OS Cell Line

Catalog Number: 93-0663C3 **Lot Number:** See Vial
Contents: 2 vials, 1 x 10⁶ cells per vial in 1 mL

Background

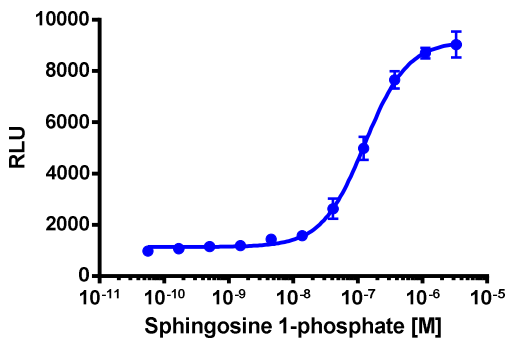
PathHunter Activated GPCR Internalization cell lines are engineered to co-express an untagged GPCR, Enzyme Acceptor (EA) tagged β -Arrestin, and a ProLink™ (PK) tag localized to the endosomes. Activation of the GPCR induces β -Arrestin recruitment, followed by internalization of the Receptor/Arrestin-EA 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: EDG3
Description: Sphingosine 1-phosphate receptor 3
Receptor Family: Lysophospholipid (S1P)
Coupling: Gq
Accession Number: NM_005226
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): RT
EC₅₀ for Agonist Stimulation (nM): 127
Signal:Background at Agonist E_{max}: 9.2

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.

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 26 Reagent	93-0563R26A
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.

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.