

# PathHunter® CHRM4 Total GPCR Internalization U2OS Cell Line

Catalog Number: 93-0902C3 Lot Number: See Vial

**Contents:** 2 vials, 1 x 10<sup>6</sup> cells per vial in 1 mL

#### **Background**

PathHunter Total GPCR Internalization cell lines are engineered to co-express the Enzyme Acceptor (EA) tagged GPCR, and a  $ProLink^{TM}$  (PK) tag localized to the endosomes. Activation of the GPCR-EA induces internalization of the receptor in PK-tagged endosomes, forcing complementation of the two  $\beta$ -galactosidase enzyme fragments (EA and PK). The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

#### **Product Information**

Target GPCR: CHRM4

**Description:** Cholinergic receptor, muscarinic 4

Receptor Family: Acetylcholine
Coupling: Gs & Gi/Go
Accession Number: NM\_000741.2

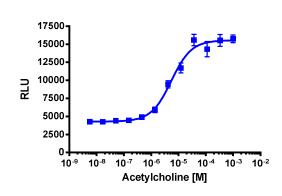
GPCR Species: Human
Cell Type: U2OS

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 <sub>2</sub> 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<sup>®</sup> Detection Kit according to the recommended protocol. Please refer to page 2 for recommended assay reagents, detection reagents, and control compounds.

Cell Number/Well:



Control Agonist:	Acetylcholine
Agonist Incubation Time (minutes):	180
Agonist Incubation Temperature (°C):	RT

 $EC_{so}$  for Agonist Stimulation (nM): 5430 Signal:Background at Agonist  $E_{max}$ : 3.6

5000



#### **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

<sup>\*</sup>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	Not Applicable	Not Applicable

## **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 <a href="mailto:info@discoverx.com">info@discoverx.com</a>. For additional information, please visit discoverx.com.



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