

## PathHunter® CHR5 Activated GPCR Internalization U2OS Cell Line

**Catalog Number:** 93-0657C3 **Lot Number:** See Vial  
**Contents:** 2 vials, 1 x 10<sup>6</sup> cells per vial in 1 mL

### Background

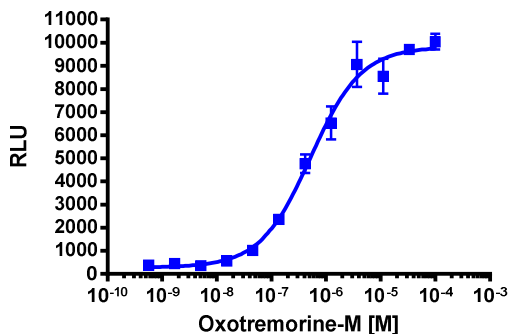
PathHunter Activated GPCR Internalization cell lines are engineered to co-express an untagged GPCR, Enzyme Acceptor (EA) tagged  $\beta$ -Arrestin, and a ProLink™ (PK) tag localized to the endosomes. Activation of the GPCR induces  $\beta$ -Arrestin recruitment, followed by internalization of the Receptor/Arrestin-EA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

### Product Information

**Target GPCR:** CHR5  
**Description:** Cholinergic receptor, muscarinic 5  
**Receptor Family:** Acetylcholine  
**Coupling:** Gq  
**Accession Number:** AF498919  
**GPCR Species:** Human  
 **$\beta$ -Arrestin Isoform:**  $\beta$ -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<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 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:** Oxotremorine-M  
**Agonist Incubation Time (minutes):** 180  
**Agonist Incubation Temperature (°C):** RT  
**EC<sub>50</sub> for Agonist Stimulation (nM):** 522.6  
**Signal:Background at Agonist E<sub>max</sub>:** 26.4

### 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<sub>50</sub>.

### 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 <sup>®</sup> 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:** Oxotremorine-M

**Vendor:** DiscoverX<sup>®</sup> (Catalog No. 92-1106)

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](mailto:info@discoverx.com). For additional information, please visit [discoverx.com](http://discoverx.com).

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