

## PathHunter® F2RL3 Activated GPCR Internalization U2OS Cell Line

**Catalog Number:** 93-0753C3

**Lot Number:** See Vial

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

### Background

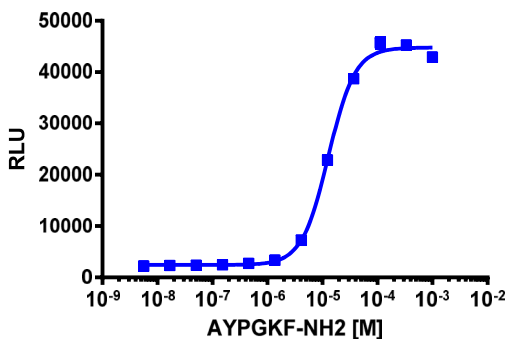
PathHunter Activated GPCR Internalization cell lines are engineered to co-express an untagged GPCR, Enzyme Acceptor (EA) tagged  $\beta$ -Arrestin, and a ProLink™ (PK) tag localized to the endosomes. Activation of the GPCR induces  $\beta$ -Arrestin recruitment, followed by internalization of the Receptor/Arrestin-EA 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

<b>Target GPCR:</b>	F2RL3
<b>Description:</b>	Coagulation factor II (thrombin) receptor-like 3 (PAR4)
<b>Receptor Family:</b>	Protease activated
<b>Coupling:</b>	Gi/Go & Gq
<b>Accession Number:</b>	AY431102
<b>GPCR Species:</b>	Human
<b><math>\beta</math>-Arrestin Isoform:</b>	$\beta$ -Arrestin-2
<b>Cell Type:</b>	U2OS
<b>Storage:</b>	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.



<b>Cell Number/Well:</b>	5000
<b>Control Agonist:</b>	Protease-Activated Receptor-4 (AYPGKF-NH2)
<b>Agonist Incubation Time (minutes):</b>	180
<b>Agonist Incubation Temperature (°C):</b>	RT
<b>EC<sub>50</sub> for Agonist Stimulation (nM):</b>	1.3 x 10 <sup>4</sup>
<b>Signal:Background at Agonist E<sub>max</sub>:</b>	19.5

### Passage Stability

This cell line has been confirmed to be stable through a minimum of 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® Detection Kit	93-0001
Cell Culture	AssayComplete™ Cell Culture Kit-103	92-3103G
Cell Plating	AssayComplete™ Cell Plating 22 Reagent	93-0563R22A
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:** Protease-Activated Receptor-4 (AYPGKF-NH2)

**Vendor:** Anaspec (Catalog No. 60218-1) or equivalent

**Ordering:** +1.510.979.1415 option 4 or e-mail [CustomerServiceDRX@eurofins.com](mailto:CustomerServiceDRX@eurofins.com)

**Technical support:** +1.510.979.1415 option 5 or e-mail [DRX\\_SupportUS@eurofinsUS.com](mailto:DRX_SupportUS@eurofinsUS.com)

**General product information:** [www.discoverx.com](http://www.discoverx.com)

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