

## PathHunter® U2OS c-Ret-GFRα1 Functional Assay

**Catalog Number:** 93-1054C3

**Lot Number:** See Vial

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

### Background

PathHunter Receptor Tyrosine Kinase (RTK) Functional cell lines are engineered to co-express a ProLink™ (PK) tagged RTK, an Enzyme Acceptor (EA) tagged SH2 domain, and, in some cases, an untagged co-receptor. Activation of the RTK-PK induces receptor dimerization, leading to SH2-EA recruitment, and forcing complementation of the two β-galactosidase enzyme fragments (EA and PK). The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

### Product Information

<b>Target RTK:</b>	c-Ret	<b>Co-receptor:</b>	GFRα1
<b>RTK Accession #:</b>	NM_020975.4	<b>Accession #:</b>	NM_001145453.1
<b>Description:</b>	Proto-oncogene tyrosine-protein kinase receptor Ret	<b>Description:</b>	GDNF family receptor alpha-1
<b>Kinase Species:</b>	Human		
<b>SH2 Domain:</b>	SHC1		
<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 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:** 10000

**Control Agonist 1:** GDNF

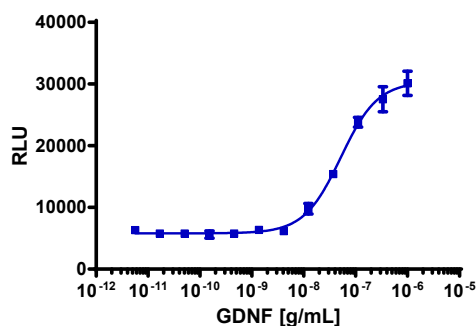
**Cell Incubation Time (hours):** 4

**Agonist Incubation Time (minutes):** Overnight

**Agonist Incubation Temperature (°C):** 37

**EC<sub>50</sub> for SH2 recruitment (ng/mL):** 51.6

**Signal:Background at agonist E<sub>max</sub>:** 5.2



### 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 <sup>®</sup> Detection Kit	93-0001
Cell Culture	AssayComplete™ Cell Culture Kit-103	92-3103G
Cell Plating	AssayComplete™ Cell Plating 16 Reagent	93-0563R16A
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 1:** GDNF

**Vendor:** Eurofins DiscoverX<sup>®</sup> (Catalog No. 92-1299)

**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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