

PathHunter[®] U2OS TrkA-P75 Functional Assay

Catalog Number: 93-0529C3

Lot Number: See Vial

Contents: 2 x 10⁶ 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.

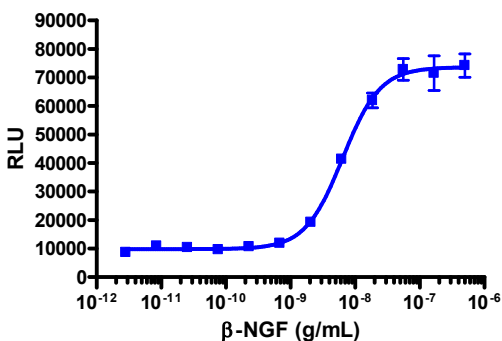
Product Information

Target RTK:	TrkA	Co-receptor:	P75
RTK Accession #:	NM_002529	Accession #:	NM_002507
Description:	Neurotrophic tyrosine kinase receptor type 1	Description:	nerve growth factor receptor
Kinase Species:	Human		
SH2 Domain:	SHC1		
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 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:	10000
Control Agonist 1:	Recombinant Human b-NGF
Cell Incubation Time (hours):	24
Agonist Incubation Time (minutes):	180
Agonist Incubation Temperature (°C):	RT
EC₅₀ for SH2 recruitment (ng/mL):	6.4
Signal:Background at agonist E_{max}:	7.4



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 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 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: Recombinant Human b-NGF

Vendor: Eurofins DiscoverX® (Catalog No. 92-1023)

Ordering: +1.510.979.1415 option 4 or e-mail CustomerServiceDRX@eurofins.com

Technical support: +1.510.979.1415 option 5 or e-mail DRX_SupportUS@eurofinsUS.com

General product information: www.discoverx.com

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