

PathHunter® U2OS EphA5 Functional Assay

Catalog Number: 93-0840C3

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: EphA5

RTK Accession #: NM_004439

Description: Ephrin type-A receptor 5

Kinase Species: Human

SH2 Domain: PLCG1

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 Ephrin-A3

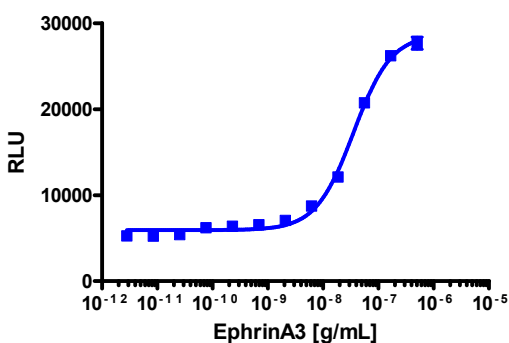
Cell Incubation Time (hours): 24

Agonist Incubation Time (minutes): 180

Agonist Incubation Temperature (°C): RT

EC₅₀ for SH2 recruitment (ng/mL): 35.6

Signal:Background at agonist E_{max}: 5.3



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	Not Applicable	Not Applicable
AssayComplete™ Hygromycin B	250	92-0029
AssayComplete™ G418	500	92-0030

Additional Ligand Information

Control Agonist 1: Recombinant Human Ephrin-A3

Vendor: Eurofins DiscoverX[®] (Catalog No. 92-1142)

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