

PathHunter® U2OS NIK Signaling Assay

Catalog Number: 93-1059C3

Lot Number: See Vial

Contents: 2 vials, 1 x 10⁶ cells per vial in 1 mL

Background

PathHunter Signaling cell lines are engineered to express an Enzyme Donor (ED) tagged target protein. Activation of the pathway results in an increase of intracellular concentration of the ED-tagged protein. Addition of exogenous Enzyme Acceptor (EA), and buffer, lyses the cell and forces complementation of the ED and EA enzyme fragments. This results in the formation of a functional enzyme that hydrolyzes substrate to generate a chemiluminescent signal.

Product Information

Target Protein: NIK

Accession #: NM_003954.3

Description: Mitogen-activated protein kinase kinase kinase 14

Target ED Tag: ePL

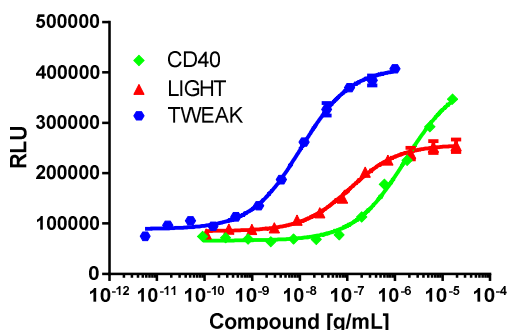
Target Species: Human

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 compound, 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
Cell Seeding Time (hours):	24
Control Agonist:	TWEAK
Ligand Incubation Time (minutes):	240
Ligand Incubation Temperature (°C):	37
EC₅₀ for compound stimulation (ng/mL):	10.6
Signal:Background at agonist E_{max}:	4.2

Assay Note: The ligands TWEAK, CD40, and LIGHT will induce an increase of signal.

Passage Stability

This cell line has been confirmed to be stable through 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® ProLabel®/ProLink™ Detection Kit	93-0812
Cell Culture	AssayComplete™ Cell Culture Kit-103	92-3103G
Cell Plating	AssayComplete™ Cell Plating 0 Reagent	93-0563R0A
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	Not Applicable	Not Applicable
AssayComplete™ G418	500	92-0030

Additional Ligand Information

Control Agonist: TWEAK

Vendor: DiscoverX® (Catalog No. 92-1297)

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. For additional information, please visit discoverx.com.

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