

PathHunter® U2OS CD137 Signaling Assay

Catalog Number: 93-1089C3

Lot Number: See Vial

Contents: 1 x 10⁶ cells per vial in 1 mL

Background

Co-stimulatory checkpoint receptor signaling assays measure activation of a member of the TNFR family of receptors, which activates the non-canonical NF-κB pathway through NF-κB inducing kinase (NIK). The cell line is engineered to express NIK labeled with enzyme donor (ED). NIK protein levels inside the cell are tightly maintained at low levels by the proteasome, and increase in response to pathway activation. Addition of exogenous Enzyme Acceptor (EA) forces complementation of the ED and EA enzyme fragments resulting in the formation of a functional β-gal enzyme that hydrolyzes substrate to generate a chemiluminescent signal. The assays may rely on either endogenous receptors or exogenously expressed target receptors.

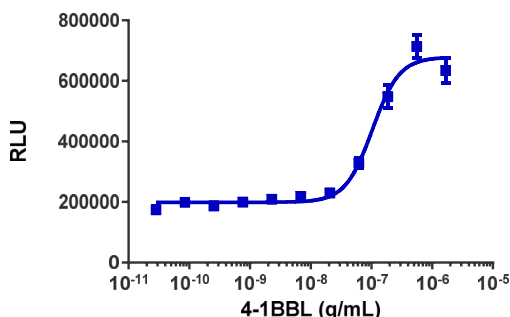
Product Information

Target Receptor:	CD137	Receptor Protein:	NIK
Expression Mode:	Engineered		
Accession #:	NM_001561.5	Accession #:	NM_003954.3
Description:	tumor necrosis factor receptor superfamily member 9	Description:	Mitogen-activated protein kinase kinase kinase 14
Target ED Tag:	None	Target ED Tag 2:	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 seeded in a 96-well plate, incubated at 37°C / 5% CO₂ followed by stimulation with a control ligand, as defined in the assay conditions below. After stimulation, assay 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.



Signaling Cell Number/Well:	25000
Signaling Cell Seeding Time (hours):	24
Control Agonist:	4-1BB-L
Ligand Incubation Time (minutes):	300
Ligand Incubation Temperature (°C):	37
EC₅₀ for compound stimulation (ng/mL):	104
Signal:Background at agonist E_{max}:	3.1

Detection Incubation Time (hrs.) 1

Longer detection time (overnight incubation at ambient temperature in the dark) can reduce replicate variability.

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 [®] 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
Ligand Dilution	AssayComplete [™] Protein Dilution Buffer	92-0023

*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	Not Applicable	Not Applicable
AssayComplete [™] G418	500	92-0030

Additional Ligand Information

Control Agonist: 4-1BB-L

Vendor: Eurofins DiscoverX[®] (Catalog No. 92-1321)

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