PathHunter® U2OS RANK/IκB Functional Assay

Catalog Number: **93-0994C3** **Lot Number:** See Vial

Contents: 1 x 10^6 cells per vial in 1 mL

Background

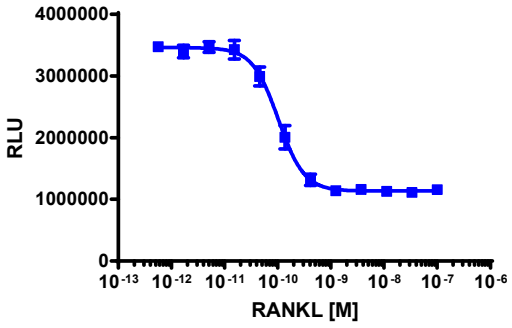
PathHunter Degradation cell lines are engineered to express an Enzyme Donor (ED) tagged target protein. Activation of the pathway results in degradation of the ED-tagged protein. Addition of exogenous Enzyme Acceptor (EA), and buffer, lyses the cell and forces complementation of the ED and EA enzyme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

Product Information

Target Protein:	RANK	**Target Protein 2:**	IκB
Accession #:	NM_003839.3	**Accession #:**	NM_020529
Description:	Tumor necrosis factor receptor superfamily, member 11a	**Description:**	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
Target ED Tag:	None	**Target ED Tag 2:**	PL
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% CO_2 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):	48
Control Agonist:	sRANKL
Ligand Incubation Time (minutes):	30
Ligand Incubation Temperature (°C):	37
EC_{50} for compound stimulation (nM):	0.102
Signal:Background at agonist E_{max}:	3.0

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_{50}.

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 38 Reagent	93-0563R38A
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	Not Applicable	Not Applicable
AssayComplete™ G418	500	92-0030

Additional Ligand Information

Control Agonist: sRANKL

Vendor: Eurofins DiscoverX® (Catalog No. 92-1282)

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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