

PathHunter® THP-1 IκB Degradation Cell Line

Catalog Number: 93-0538C14

Lot Number: See Vial

Contents: 2 vials, 1 x 10⁶ 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: IκB

Accession #: NM_020529

Description: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha

Target ED Tag: PL

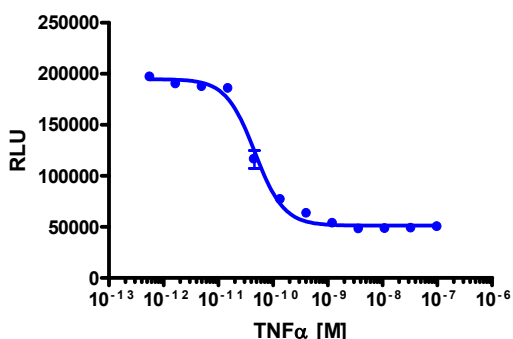
Target Species: Human

Cell Type: THP-1

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): 0

Control Agonist: TNFα (human)

Ligand Incubation Time (minutes): 60

Ligand Incubation Temperature (°C): 37

EC₅₀ for compound stimulation (nM): 0.05

Signal:Background at agonist E_{max}: 3.9

For optimal growth of PathHunter THP-1 IκB cells, we recommend supplementing growth and recovery media with β-mercaptoethanol to a final concentration of 0.05mM.

Important! Cell handling/protocol notes: THP-1 is a suspension cell line. Do not aspirate media prior to propagation or cell culture will be lost. To propagate, transfer a fraction of the cell suspension to a new flask containing fresh growth media. Cell suspension may need to be concentrated (pelleted and re-suspended) prior to loading on assay plate; compounds can be added any time after cell plating, but plates should incubate at 37°C/5% CO₂ until that time.

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-101	92-3101G
Cell Plating	AssayComplete [™] Cell Plating 25 Reagent	93-0563R25A
Cell Detachment	AssayComplete [™] Cell Detachment Reagent	92-0009
Cell Thawing	AssayComplete [™] Thawing Reagent T6	92-4106TR
Cell Freezing	AssayComplete [™] Freezing Reagent F5	92-5105FR

*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	300	92-0030

Additional Ligand Information

Control Agonist: TNF α (human)

Vendor: DiscoverX[®] (Catalog No. 92-1097)

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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