

PathHunter® U2OS Keap1-NRF2 Nuclear Translocation Cell Line

Catalog Number: 93-0821C3 **Lot Number:** See Vial
Contents: 2 vials, 1 x 10⁶ cells per vial in 1 mL

Background

PathHunter® Nuclear Translocation cell lines are engineered to co-express two proteins: a) Enzyme Donor (ED) tagged target protein; b) an Enzyme Acceptor (EA), which is localized to the nucleus. Depending on the assay, activation of the signaling pathway can either a) induce translocation of the ED-tagged target protein into the nucleus, which will force complementation of the two enzyme fragments, and result in the formation of a functional enzyme, that will hydrolyze substrate and generate a chemiluminescent signal; or b) induce the ED-tagged protein to vacate the nucleus, resulting in a decrease of functional enzyme and a subsequent decrease of chemiluminescent signal. Some nuclear translocation assays will also co-express an untagged secondary protein involved in the pathway of interest.

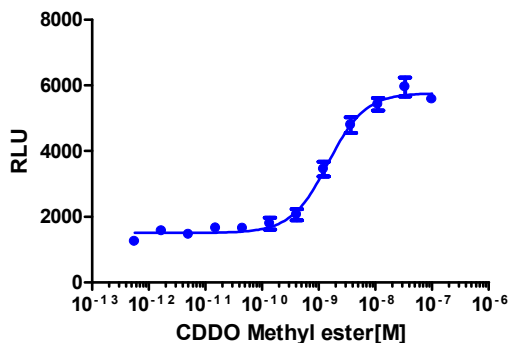
Product Information

Translocating Protein:	NRF2	Secondary Protein:	Keap1
Accession #:	NM_203500.1	Accession #:	NM_006164
Description:	kelch-like ECH-associated protein 1	Description:	nuclear factor (erythroid-derived 2)-like 2

ED Tag: PK2
Protein 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 overnight 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
Control Compound:	CDDO Methyl Ester
Compound Incubation Time (minutes):	360
Compound Incubation Temperature (°C):	RT
EC₅₀ for Compound Stimulation (nM):	1.4
Signal:Background at Compound E_{max}:	4.4

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® Detection Kit	93-0001
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	0.25	92-0028
AssayComplete™ Hygromycin B	250	92-0029
AssayComplete™ G418	500	92-0030

Additional Ligand Information

Control Compound: CDDO Methyl Ester

Vendor: DiscoverX® (Catalog No. 92-1147)

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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