

PathHunter® eXpress Keap1-NRF2 Nuclear Translocation Assay

Catalog Number: 93-0821E3

Lot Number: See Vial

Contents: 1 x 10⁶ cells per vial in 0.1 mL

Background

PathHunter® Nuclear Translocation cells 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. These cells have been modified to prevent long term propagation and expansion using a proprietary compound that has no apparent effect on assay performance.

Product Information

Translocating Protein: NRF2

Secondary Protein: Keap1

Accession #: NM_006164

Accession #: NM_203500.1

Description: nuclear factor (erythroid-derived 2)-like 2

Description: Kelch-like ECH-associated Protein-1

ED Tag: PK2

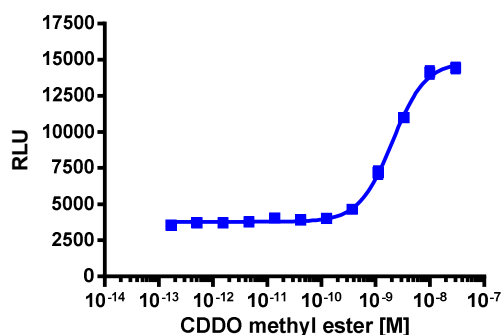
Protein Species: Human

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 96-well plate and incubated at 37°C and 5% CO₂. Cells were then stimulated with a control compound, using the assay conditions described below. Following stimulation, signal was detected using the PathHunter Detection Reagents provided in the kit according to the recommended protocol. For a detailed protocol, please refer to the user manual.



Cell Number/Well: 10000

Cell Plating Reagent: AssayComplete™ Cell Plating 0 Reagent

Control Compound: CDDO Methyl Ester

Cell Incubation Time (Hours): 24

Compound Incubation Time (minutes): 360

Compound Incubation Temperature (°C): RT

EC₅₀ for Compound Stimulation: 2

Signal:Background at Compound E_{max}: 4.1

Additional Ligand Information

Control Compound: CDDO Methyl Ester

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

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