

## PathHunter® eXpress FOXO3 Nuclear Translocation Assay

**Catalog Number:** 93-0876E3

**Lot Number:** See Vial

**Contents:** 1 x 10<sup>6</sup> 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:** FOXO3

**Accession #:** NM\_201559.2

**Description:** forkhead box O3

**ED Tag:** PK2

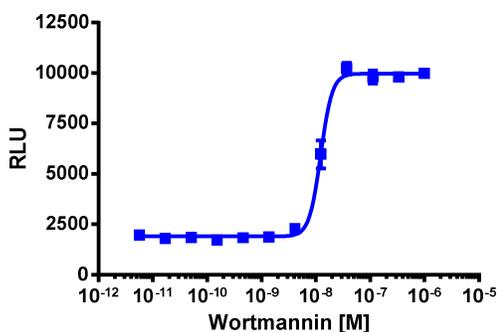
**Protein Species:** Human

**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% CO<sub>2</sub>. 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 5 Reagent

**Control Compound:** Wortmannin

**Cell Incubation Time (Hours):** 24

**Compound Incubation Time (minutes):** 120

**Compound Incubation Temperature (°C):** 37

**EC<sub>50</sub> for Compound Stimulation:** 12.3

**Signal:Background at Compound E<sub>max</sub>:** 5.2

### Additional Ligand Information

**Control Compound:** Wortmannin

**Vendor:** Eurofins DiscoverX<sup>®</sup> (Catalog No. 92-1095)

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