

PathHunter[®] U2OS mRHO(P23H) ENDO-EA Pharmacotraficking Assay

Catalog Number: 93-0990C3 **Lot Number:** See Vial

Contents: 2 vials, 1 x 10⁶ cells per vial in 1 mL

Background

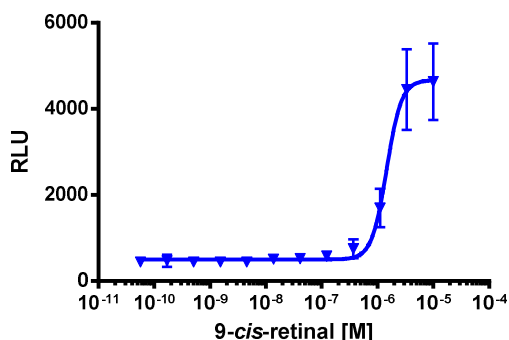
PathHunter Pharmacotraficking cell lines are engineered to co-express a ProLink™ (PK)-tagged transmembrane protein retained in the ER (due to protein misfolding), and an Enzyme Acceptor (EA) tag localized to the endosomes. Binding of a chemical pharmacochaperone to the misfolded, PK-tagged protein stabilizes the protein in a conformation that enables its trafficking through the Golgi, then onward to the cell membrane. Protein re-localized to the cell membrane subsequently internalizes (either passively or actively) into EA-tagged endosomes, forcing complementation of the two β-galactosidase enzyme fragments (EA and PK). The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

Product Information

Target Protein: mRHO(P23H)
Description: Rhodopsin
Accession Number: NM_145383.1
Target Species: Mouse
ProLink™ Tag: PK1
Readout: Endosome - EA
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 for at least 4 hours at 37°C and 5% CO₂ to allow the cells to attach. 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: 9-cis-retinal
Compound Incubation Time (minutes): Overnight
Compound Incubation Temperature (°C): 37
EC₅₀ for Compound Stimulation (nM): 1460
Signal:Background at Agonist E_{max}: 8.7

Important Note: mRHO (NM_145383.1) mutated to mRHO(P23H).

Additional Protocol Information: After completion of overnight compound treatment @ 37° C: Remove assay plate from incubator and incubate an additional 3 hours at room temperature, then add PathHunter[®] Detection Reagent.

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 5 Reagent	93-0563R5A
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	Not Applicable	Not Applicable
AssayComplete™ Hygromycin B	250	92-0029
AssayComplete™ G418	500	92-0030

Additional Ligand Information

Control Compound: 9-*cis*-retinal

Vendor: DiscoverX® (Catalog No. 92-1280)

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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