

## PathHunter<sup>®</sup> U2OS MC4R(T162I) ENDO-EA Pharmacotraficking Assay

**Catalog Number:** 93-1004C3 **Lot Number:** See Vial

**Contents:** 2 vials, 1 x 10<sup>6</sup> cells per vial in 1 mL

### Background

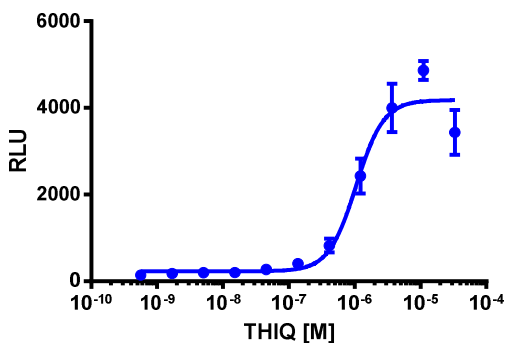
PathHunter Pharmacotraficking cell lines are engineered to co-express a ProLink™ (PK)-tagged transmembrane protein retained in the ER (due to protein misfolding), and an Enzyme Acceptor (EA) tag localized to the endosomes. Binding of a chemical pharmacochaperone to the misfolded, PK-tagged protein stabilizes 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:** MC4R(T162I)  
**Description:** Melanocortin receptor 4  
**Accession Number:** NM\_005912.2  
**Target Species:** Human  
**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% CO<sub>2</sub> 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:** THIQ  
**Compound Incubation Time (minutes):** Overnight  
**Compound Incubation Temperature (°C):** 37  
**EC<sub>50</sub> for Compound Stimulation (nM):** 1050  
**Signal:Background at Agonist E<sub>max</sub>:** 34.7

**Important Note:** MC4R (NM\_005912.2) mutated to MC4R(T162I).

### Passage Stability

This cell line has been confirmed to be stable through 10 passages with no significant drop in assay window or change in EC<sub>50</sub>.

### 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:** THIQ

**Vendor:** DiscoverX® (Catalog No. 92-1278)

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](mailto:info@discoverx.com). For additional information, please visit [discoverx.com](http://discoverx.com).

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