PathHunter® U2OS ENDO-EA Parental Cell Line

Catalog Number: **93-1102C3** **Lot Number:** See Vial

Contents: 2 vials, 2 x 10^6 cells per vial in 1 mL

Background

PathHunter translocation EA parental cell lines use DiscoverX's proprietary enzyme fragment complementation (EFC) technology. The cell lines are engineered to express a large fragment of β-galactosidase (β-gal) called enzyme acceptor (EA) fused to a reporter protein (Reporter-EA) which is localized to a specific subcellular compartment. Introduction of a smaller fragment of β-gal called ED [ProLabel (PL) or ProLink (PK)] fused to a target protein into EA cells allows for development of assays to monitor translocation of the target protein into the reporter protein 'labeled' subcellular compartment. Assays using these cell lines can be used to screen and study ligands (e.g. agonists/antagonists, pharmacological chaperones, etc.) or potentially other experimental manipulations that results in translocation of the target protein to the compartment where the Reporter-EA resides. Co-localization of these two proteins forces complementation of the two β-gal enzyme fragments (EA and ED), which results in a functional enzyme which hydrolyzes PathHunter detection reagents to generate a chemiluminescent signal.

Product Information

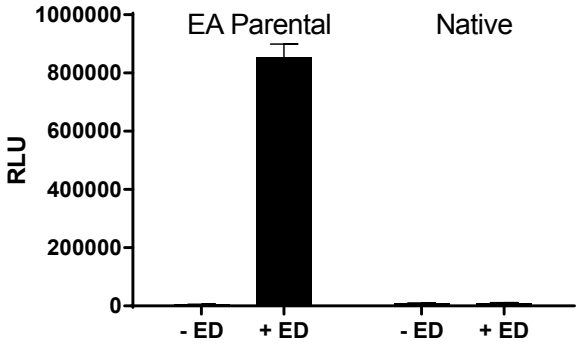
Expressed Protein: ENDO-EA

Tag Compartment:

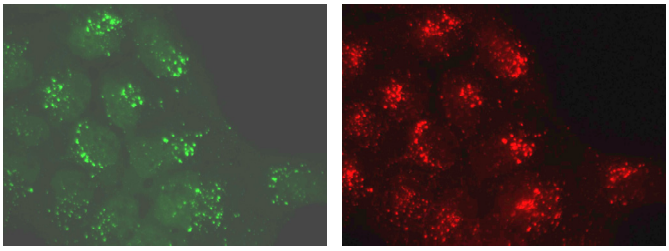
Cell Type: U2OS

Storage: Short term (<24 h): Store at -80°C; Long term (>24 h): Store in vapor phase of liquid nitrogen.

Functional Performance



EA Parental or native cells were seeded in a 384-well plate and incubated overnight at 37°C/5% CO2. Following cell lysis in the absence (left bar) and presence (right bar) of excess Enzyme Donor (ED or PK), β-galactosidase luminescence signal was detected using the PathHunter Detection Kit according to the recommended protocol. Please refer to page 2 for recommended assay and detection reagents and control compounds. Data are plotted as RLU (mean ± standard deviation).



U2OS ENDO-EA Parental cells were stained with anti-EA (green; left panel) and anti-EEA1 (red; right panel). EEA1 is early endosome antigen 1 localized to endosomes (Note: the epitope sequence recognized by the anti-EEA1 antibody used (part. no. c45b10; Cell Signaling Technologies) are not present in the ENDO-EA reporter protein sequence). The colocalization of green and red staining in the cells demonstrates that expression of ENDO-EA protein is expressed in early endosomes.

Passage Stability

This cell line has been confirmed to stably express the EA-fusion reporter protein through 10 passages.

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 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	Not Applicable	Not Applicable

ProLink™ Vectors (minimum one required)

Product Description	Catalog Number
pCMV-ProLink 1 Vector	93-0167
pCMV-ProLink 2 Vector	93-0171

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