

PathHunter® HEK 293 β -Arrestin2-EA Parental Cell Line

Catalog Number: 93-0165

Lot Number:

See Vial

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

Background

PathHunter β -arrestin1 and 2 EA parental cell lines allow for introduction of your own GPCRs (or other β -arrestin binding protein) and development of your own β -arrestin recruitment assays. PathHunter β -arrestin assays take advantage of DiscoverX's proprietary enzyme fragment complementation technology. The GPCR (or other β -arrestin binding protein) is fused in frame with a small enzyme donor fragment of β -galactosidase (β -gal) called ProLink™ (PK) which is then co-expressed in the EA parental cell line stably expressing a fusion protein of β -arrestin with a larger fragment of β -gal called enzyme acceptor (EA). Activation of the GPCR-PK (or other PK-tagged β -arrestin binding protein), which induces binding to the β -arrestin-EA fusion protein, forces complementation of the two β -gal enzyme fragments, resulting in the formation of an active β -gal enzyme. Addition of PathHunter detection reagents results in generation of a chemiluminescent signal, allowing the interaction of β -arrestin with GPCR or β -arrestin binding protein to be detected.

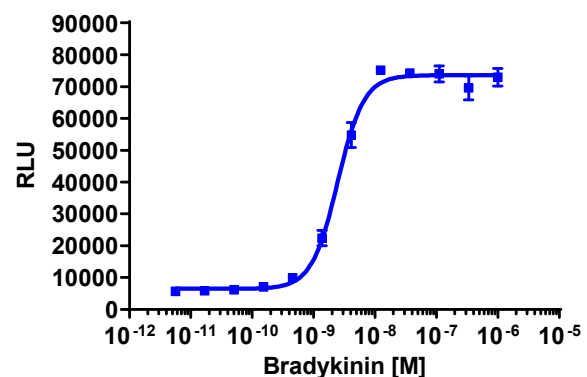
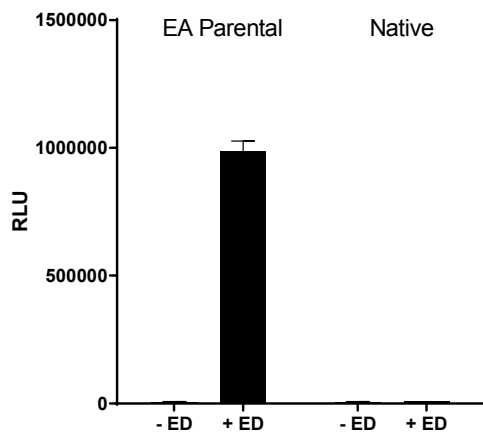
Product Information

Expressed Protein: β -Arrestin2-EA

Cell Type: HEK 293

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 $37^{\circ}\text{C}/5\% \text{CO}_2$. 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 \pm standard deviation).

Representative Data: A stable pool of β -arrestin2-EA parental cells expressing PK-tagged receptor (BDKRB2; NM_000623.3) were plated in a multi-well plate and stimulated with a control agonist (Bradykinin), using the following assay conditions: Cell Plating Reagent: AssayComplete™ Cell Plating 0 Reagent; Cell Incubation Time (Hours): 48h; Agonist Incubation Time (Minutes): 90; Agonist Incubation Temperature ($^{\circ}\text{C}$): 37. EC_{50} 2.5 nM; S:B 11

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-105	92-3105G
Cell Detachment	AssayComplete™ Cell Detachment Reagent	92-0009
Cell Thawing	AssayComplete™ Thawing Reagent T1	92-4101TR
Cell Freezing	AssayComplete™ Freezing Reagent F1	92-5101FR

*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	200	92-0029
AssayComplete™ G418	Not Applicable	Not Applicable

ProLink™ Vectors (minimum one required)

Product Description	Catalog Number
pCMV-ProLink™ Cloning Vector Bundle (contains all 4 PK vectors)	93-0491
pCMV-ProLink™ 1 Vector	93-0167
pCMV-ProLink™ 2 Vector	93-0171
pCMV-ARMS1-ProLink™ 2 Vector	93-0489
pCMV-ARMS2-ProLink™ 2 Vector	93-0490

For order placement or technical support, please call 1.510.771.3500 (North America) +44.121.260.6142 (Europe) or e-mail info@discoverx.com. For additional information, please visit discoverx.com.

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