

## PathHunter<sup>®</sup> U2OS $\beta$ -Arrestin2-EA Parental Cell Line

**Catalog Number:** 93-0166

**Lot Number:**

See Vial

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

### Background

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

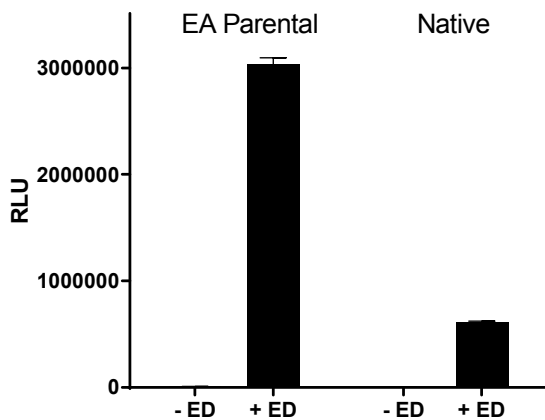
### Product Information

**Expressed Protein:**  $\beta$ -Arrestin2-EA

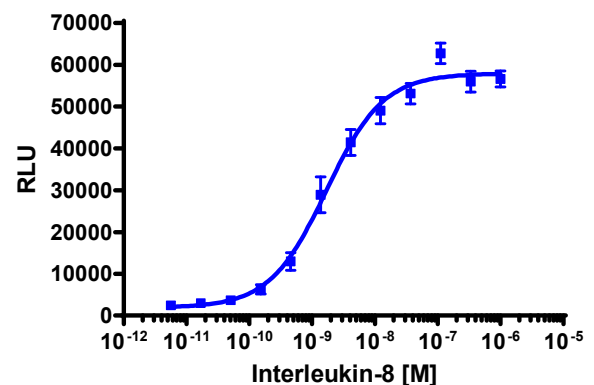
**Cell Type:** U2OS

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

### Functional Performance



EA Parental or native cells were seeded in a 384-well plate and incubated overnight at 37°C/5% CO<sub>2</sub>. Following cell lysis in the absence (left bar) and presence (right bar) of excess Enzyme Donor (ED or PK),  $\beta$ -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: CXCR1 (IL8RA; NM\_000634) tagged with PK1 was introduced into  $\beta$ Arrestin2-EA parental cells. Cell stably expressing CXCR1-PK1 and  $\beta$ Arrestin2-EA were seeded in a multi-well plate and stimulated with Interleukin -8, using the following assay conditions: Agonist Incubation Time (Minutes): 90; Agonist Incubation Temperature (°C): 37. EC<sub>50</sub> 1.3nM; S:B 14.7

### 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 <sup>®</sup> 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™ 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](mailto:info@discoverx.com). For additional information, please visit [discoverx.com](http://discoverx.com).

## Limited Use License Agreement

These products may be covered by issued US and/or foreign patents, patent application and subject to Limited Use Label License.

Please visit [discoverx.com/license](https://discoverx.com/license) for a list of products that are governed by limited use label license terms and relevant patent and trademark information.