

PathHunter® U2OS SHC1-EA Parental Cell Line

Catalog Number: 93-1123C3

Lot Number:

See Vial

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

Background

PathHunter SH2 Recruitment EA Parental Cell Lines express EA fused to the SH2 domains of adaptor proteins, like SHC1 or PLCG1. These cell lines can be used to build your own receptor tyrosine kinase (RTK), cytokine receptor kinase, or cytosolic tyrosine kinase (CTK) functional assay cell lines to monitor activation of these receptors or tyrosine kinases of interest in your research. To develop a functional assay cell line, a researcher will introduce a PK-tagged receptor, and, in some cases, an untagged co-receptor, into one of these two parental cell lines. Activation of the receptor-PK induces receptor tyrosine phosphorylation, leading to SH2-EA recruitment, and forcing complementation of the two β -galactosidase enzyme fragments (EA and PK). The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

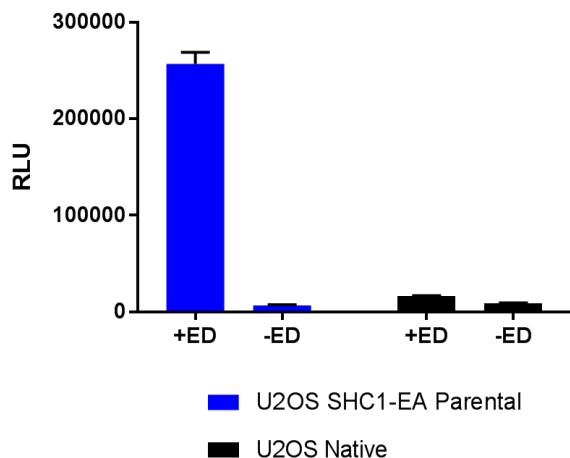
Product Information

Expressed Protein: SHC1(SH2)-EA

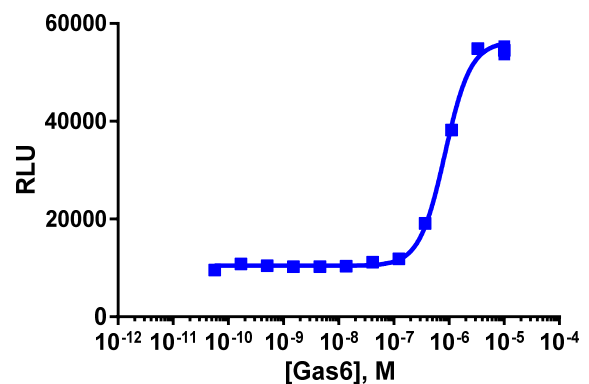
Cell Type: U2OS

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% 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 \pm standard deviation). Signal:Background (+ED/-ED) was 42.6 and ~1 for the U2OS SH2 parental and native U2OS cells, respectively.



Representative Data: TYRO3 (NM_006293.3) tagged with PK1 was introduced into U2OS SHC1-EA parental cells. Cells stably co-expressing TYRO3-PK1 and SHC1(SH2)-EA were seeded in a 384 well plate and stimulated with GAS6, using the following assay conditions: Cells seeding: 10,000/well in 384 well plate; Agonist Incubation: 360 min at 37°C. The observed EC₅₀ was 845 ng/mL and signal-to-background (S:B) was 5.6.

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™ Vector (one required)

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

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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