PathHunter® U2OS YES1 Activity Assay

Catalog Number: **93-0883C3** **Lot Number:** See Vial

Contents: 2 vials, 4 x 10^6 cells per vial in 1 mL

Background

PathHunter Cytosolic Tyrosine Kinase (CTK) Activity cell lines are engineered to express the catalytic domain from CTK of interest fused to the cytokine receptor binding domain (CRBD) of a JAK kinase. The cells co-express an Enzyme Acceptor (EA) tagged SH2 domain and a ProLink™ (PK) tagged Cytokine receptor. The CRBD is designed to bind the CTK catalytic domain to the receptor-PK. Activation of the cytokine receptor-PK induces receptor phosphorylation by the CTK catalytic domain. This leads to SH2-EA recruitment, 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

CTK Kinase Domain: YES1 [Accession #: NM_005433.3] Amino Acid Range: (aa 277 - 543)

Description: v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1

CRBD: JAK2 [Accession #: BC039695] Amino Acid Range: (aa 1 - 824)

CTK Receptor GHR [Accession #: NM_000163]

Species: Human

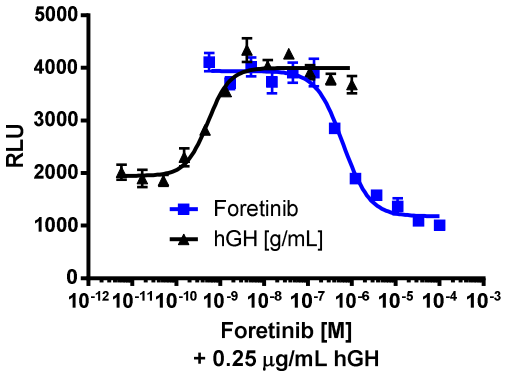
SH2 Domain: PLCG1

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 overnight at 37°C and 5% CO_2 to allow the cells to attach and grow. Cells were then pre-incubated with inhibitor, followed by stimulation with the control agonist 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 Inhibitor:	Foretinib
Inhibitor Pre-incubation Time (minutes):	120
Inhibitor Pre-incubation Temperature (°C):	37
IC_{50} for the Inhibitor (nM):	634
Signal:Background of the Inhibitor:	4.1
Control Agonist:	hGH
Agonist Incubation Time (minutes):	180
Agonist Incubation Temperature (°C):	RT

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_{50}.

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 16 Reagent	93-0563R16A
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	0.25	92-0028
AssayComplete™ Hygromycin B	250	92-0029
AssayComplete™ G418	500	92-0030

Additional Ligand Information

Control Agonist: hGH

Vendor: DiscoverX® (Catalog No. 92-1338)

Control Inhibitor Foretinib

Vendor: DiscoveRx® (Catalog No. 92-1238)

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. For additional information, please visit discoverx.com.

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