

PathHunter® eXpress BLK Activity Assay

Catalog Number: 93-0882E3

Lot Number:

See Vial

Contents: 1 x 10⁶ cells per vial in 0.1 mL

Background

PathHunter 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. These cells have been modified to prevent long term propagation and expansion using a proprietary compound that has no apparent effect on assay performance.

Product Information

Target Kinase Domain: BLK Amino Acid Range: (aa 241 - 506)

Description: B lymphoid tyrosine kinase

Target Accession #: NM_001715.2

Species: Human

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

SH2 Domain: PLCG1

Cell Type: U2OS

Cell Plating Reagent: AssayComplete™ Cell Plating 16 Reagent

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 96-well plate and incubated for 24 or 48 hours at 37°C and 5% CO₂. Cells were then pre-incubated with inhibitor, followed by stimulation with the control agonist using the assay conditions described below. Following agonist stimulation, signal was detected using the PathHunter Detection Reagents provided in the kit according to the recommended protocol. For a detailed protocol, please refer to the user manual.

Control Agonist: Recombinant Human Growth Hormone

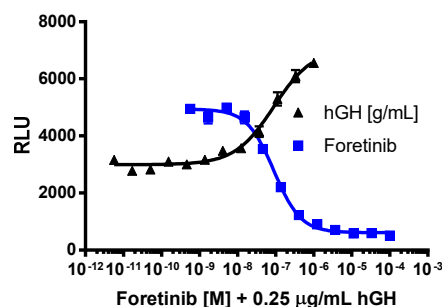
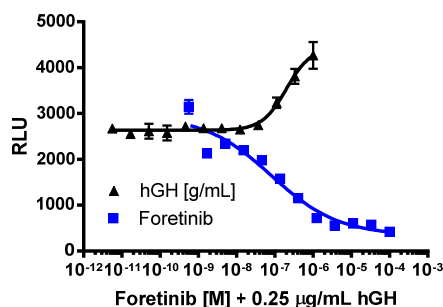
Control Inhibitor: Foretinib

Agonist Incubation Time (minutes): 180

Inhibitor Pre-incubation Time (minutes): 120

Agonist Incubation Temperature (°C): RT

Inhibitor Pre-incubation Temperature (°C): 37

24 Hours
48 Hours

IC₅₀ for the Inhibitor (nM): 81

IC₅₀ for the Inhibitor (nM): 90

Signal:Background of the Inhibitor: 7.4

Signal:Background of the Inhibitor: 9.8

Additional Information

Control Agonist: Recombinant Human Growth Hormone

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

Control Inhibitor Foretinib

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

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