

## PathHunter® eXpress SYK Activity Assay

**Catalog Number:** 93-0865E3

**Lot Number:**

See Vial

**Contents:** 1 x 10<sup>6</sup> 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:** SYK Amino Acid Range: (aa 371 - 636)

**Description:** Spleen tyrosine kinase

**Target Accession #:** NM\_003177.5

**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% CO<sub>2</sub>. 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.

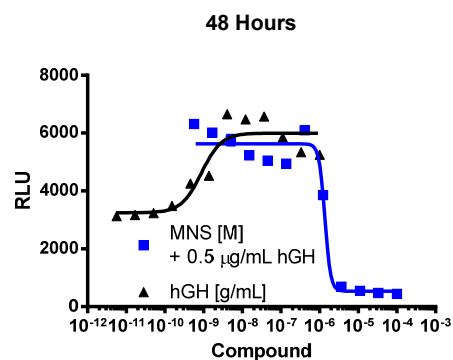
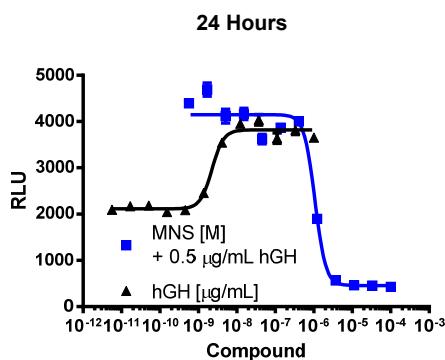
**Control Agonist:** Recombinant Human Growth Hormone

**Control Inhibitor:** MNS

**Agonist Incubation Time (minutes):** 180

**Inhibitor Pre-incubation Time (minutes):** 120

**Agonist Incubation Temperature (°C):** RT

**Inhibitor Pre-incubation Temperature (°C):** 37

**IC<sub>50</sub> for the Inhibitor (nM):** 1068

**IC<sub>50</sub> for the Inhibitor (nM):** 1371

**Signal:Background of the Inhibitor:** 10.3

**Signal:Background of the Inhibitor:** 14.2

### Additional Information

**Control Agonist:** Recombinant Human Growth Hormone

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

**Control Inhibitor** MNS

**Vendor:** DiscoverX® (Catalog No. 2877)

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