

## PathHunter® eXpress FLT3 Activity Assay

**Catalog Number:** 93-0901E3

**Lot Number:**

See Vial

**Contents:** 1 x 10<sup>6</sup> cells per vial in 0.1 mL

### Background

PathHunter RTK Activity cell lines are engineered to express a fusion of the intracellular catalytic domain from the target RTK and the extracellular ligand binding domain of EGFR. This fusion protein is tagged with ProLink™ (PK). The cells co-express an Enzyme Acceptor (EA) tagged SH2 domain. Ligand induced cross-phosphorylation of the PK-tagged fusion protein 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:** FLT3 Amino Acid Range: (aa 540 - 993)

**Description:** Fms-related tyrosine kinase 3

**Target Accession #:** NM\_004119

**Kinase Species:** Human

**RTK Membrane Domain:** tEGFR [Accession #: NM\_005228.3] Amino Acid Range: (aa 1 - 675)

**SH2 Domain:** PLCG2

**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 EGF

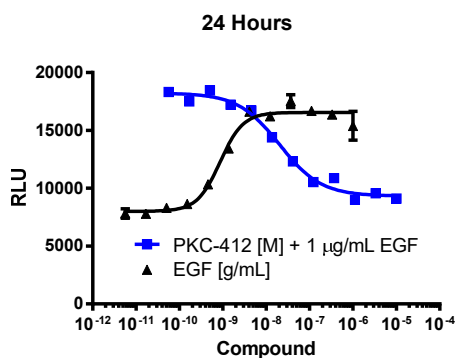
**Agonist Incubation Time (minutes):** 180

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

**Control Inhibitor:** PKC-412

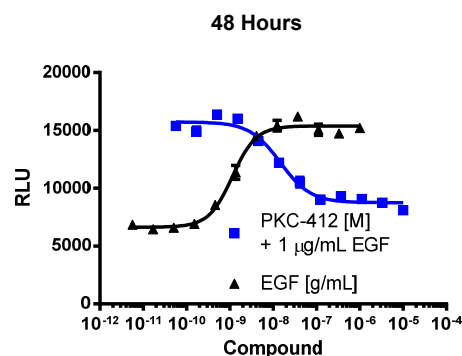
**Inhibitor Pre-incubation Time (minutes):** 60

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



**IC<sub>50</sub> for the Inhibitor (nM):** 20.3

**Signal:Background of the Inhibitor:** 2.0



**IC<sub>50</sub> for the Inhibitor (nM):** 14.5

**Signal:Background of the Inhibitor:** 2.0

Signal:Background of the inhibitor:

Signal:Background of the inhibitor:

### Additional Information

**Control Agonist:** Recombinant Human EGF

**Vendor:** Eurofins DiscoverX® (Catalog No. 92-1113)

**Control Inhibitor** PKC-412

**Vendor:** DiscoverX® (Catalog No. M1323)

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