

PathHunter® eXpress FLT4 Activity Assay

Catalog Number: 93-0888E3

Lot Number:

See Vial

Contents: 1 x 10⁶ 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: FLT4 Amino Acid Range: (aa 776 - 1363)

Description: fms-related tyrosine kinase 4

Target Accession #: NM_182925

Kinase Species: Human

RTK Membrane Domain: tEGFR [Accession #: NM_005228.3] Amino Acid Range: (aa 1 - 645)

SH2 Domain: GRB2

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.

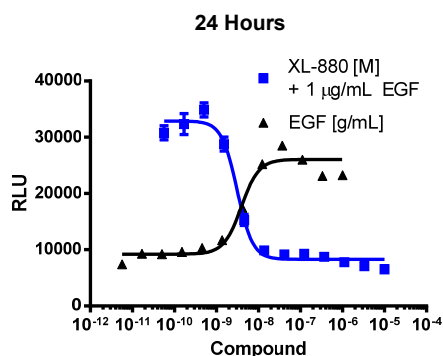
Control Agonist: Recombinant Human EGF

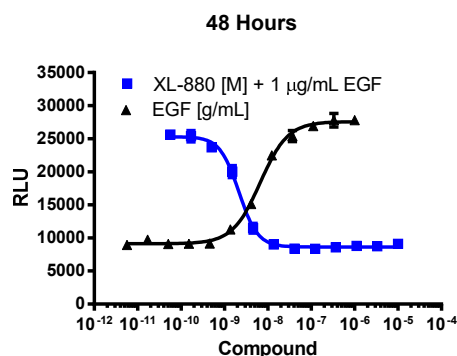
Agonist Incubation Time (minutes): 180

Agonist Incubation Temperature (°C): RT

Control Inhibitor: Foretinib

Inhibitor Pre-incubation Time (minutes): 60

Inhibitor Pre-incubation Temperature (°C): 37

IC₅₀ for the Inhibitor (nM): 3.1

Signal:Background of the Inhibitor: 4.5

IC₅₀ for the Inhibitor (nM): 2.2

Signal:Background of the Inhibitor: 2.8

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 Foretinib

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

Limited Use License Agreement

These products may be covered by issued US and/or foreign patents, patent application and subject to Limited Use Label License.

Please visit discoverx.com/license for a list of products that are governed by limited use label license terms and relevant patent and trademark information.

Ordering: +1.510.979.1415 option 4 or e-mail CustomerServiceDRX@eurofins.com

Technical support: +1.510.979.1415 option 5 or e-mail DRX_SupportUS@eurofinsUS.com

General product information: www.discoverx.com