

PathHunter® eXpress KDR Activity Assay

Catalog Number: 93-0903E3

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: KDR Amino Acid Range: (aa 763 - 1356)

Description: Vascular endothelial growth factor receptor 2

Target Accession #: NM_002253

Kinase Species: Human

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

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 EGF

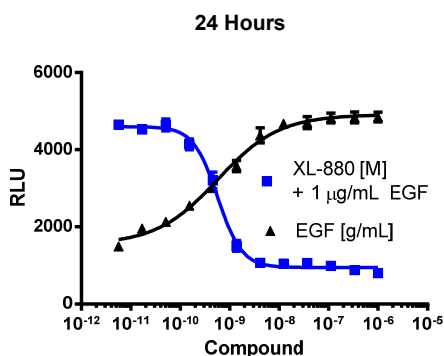
Agonist Incubation Time (minutes): 180

Agonist Incubation Temperature (°C): RT

Control Inhibitor: Foretinib

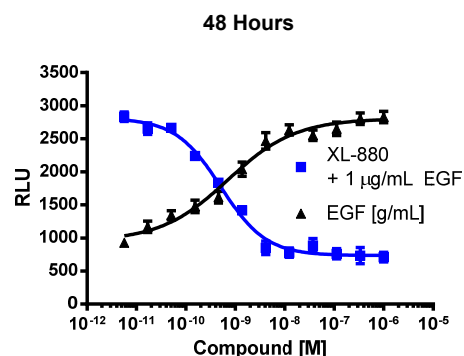
Inhibitor Pre-incubation Time (minutes): 60

Inhibitor Pre-incubation Temperature (°C): 37



IC₅₀ for the Inhibitor (nM): 0.06

Signal:Background of the Inhibitor: 5.8



IC₅₀ for the Inhibitor (nM): 0.5

Signal:Background of the Inhibitor: 3.9

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)

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