

## PathHunter® U2OS FLT3 Activity Assay

**Catalog Number:** 93-0901C3 **Lot Number:** See Vial  
**Contents:** 2 vials, 4 x 10<sup>6</sup> cells per vial in 1 mL

### Background

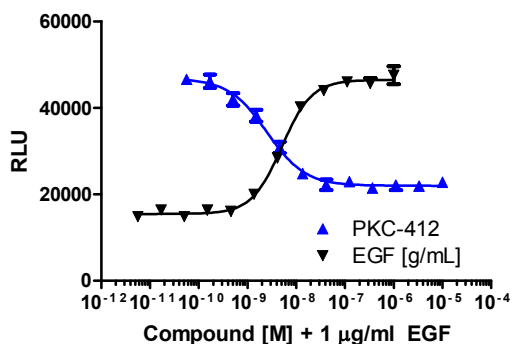
PathHunter Receptor Tyrosine Kinase (RTK) Activity cell lines are engineered to express a fusion of the intracellular catalytic domain from the target RTK and the extracellular ligand binding domain of EGFR. This fusion protein is tagged with ProLink™ (PK). The cells co-express an Enzyme Acceptor (EA) 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.

### Product Information

**Target Kinase Domain:** FLT3 Amino Acid Range: (aa 540 - 993)  
**Description:** Fms-related tyrosine kinase 3  
**Target Accession #:** NM\_004119  
**Species:** Human  
**Ligand Binding Domain:** tEGFR [Accession #: NM\_005228.3] Amino Acid Range: (aa 1 - 645)  
**SH2 Domain:** PLCG2  
**Cell Type:** U2OS  
**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 384-well plate and incubated at 37°C and 5% CO<sub>2</sub> to allow the cells to attach and grow. Cells were then pre-incubated with inhibitor, followed by stimulation with the control agonist using the assay conditions described below. Following stimulation, signal was detected using the PathHunter Detection Kit according to the recommended protocol. Please refer to page 2 for recommended assay reagents, detection reagents, and control compounds.



**Cell Number/Well:** 10000  
**Cell Incubation Time (hours):** 24  
**Control Inhibitor:** PKC-412  
**Inhibitor Pre-incubation Time (minutes):** 60  
**Inhibitor Pre-incubation Temperature (°C):** 37  
**IC<sub>50</sub> for the Inhibitor (nM):** 2.5  
**Signal:Background of the Inhibitor:** 2.1  
**Control Agonist:** EGF  
**Agonist Incubation Time (minutes):** 180  
**Agonist Incubation Temperature (°C):** RT

### Passage Stability

This cell line has been confirmed to be stable through 10 passages with no significant drop in assay window or change in EC<sub>50</sub>.

### Mycoplasma Testing

This lot was tested and found to be free of mycoplasma contamination. Data available upon request.

### Required Materials

The following additional materials are required but not provided:

Product Use*	Product Description	Catalog Number
Detection	PathHunter® Detection Kit	93-0001
Cell Culture	AssayComplete™ Cell Culture Kit-103	92-3103G
Cell Plating	AssayComplete™ Cell Plating 16 Reagent	93-0563R16A
Cell Detachment	AssayComplete™ Cell Detachment Reagent	92-0009
Cell Thawing	AssayComplete™ Thawing Reagent T3	92-4103TR
Cell Freezing	AssayComplete™ Freezing Reagent F3	92-5103FR

\*Please inquire about our cell line-specific AssayComplete Starter Packs to get you started with your cell culture needs.

### Required Antibiotics

Antibiotic Name	Concentration (µg/mL)	Catalog Number
AssayComplete™ Puromycin	Not Applicable	Not Applicable
AssayComplete™ Hygromycin B	250	92-0029
AssayComplete™ G418	500	92-0030

### Additional Compound Information

**Control Agonist:** EGF

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

**Control Inhibitor** PKC-412

**Vendor:** SelleckChem (Catalog No. S1111)

For order placement or technical support, please call 1.866.448.4864 (North America) or +44.121.260.6142 (Europe) or e-mail [info@discoverx.com](mailto:info@discoverx.com). For additional information, please visit [discoverx.com](http://discoverx.com).

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