

PathHunter® U2OS ErbB2-ErbB3 Functional Assay

Catalog Number: 93-0535C3

Lot Number: See Vial

Contents: 2 x 10⁶ cells per vial in 1 mL

Background

PathHunter Receptor Tyrosine Kinase (RTK) Functional cell lines are engineered to co-express a ProLink™ (PK) tagged RTK, an Enzyme Acceptor (EA) tagged SH2 domain, and, in some cases, an untagged co-receptor. Activation of the RTK-PK induces receptor dimerization, leading to SH2-EA recruitment, and forcing complementation of the two β-galactosidase enzyme fragments (EA and PK). The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

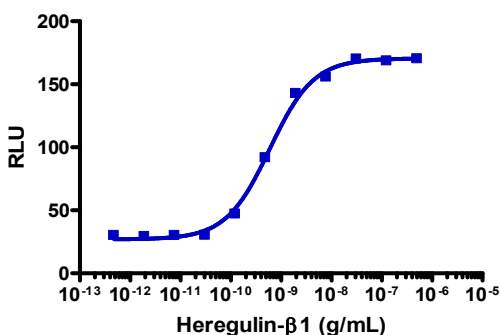
Product Information

| | | | |
|-------------------------|---|---------------------|---|
| Target RTK: | ErbB2 | Co-receptor: | ErbB3 |
| RTK Accession #: | NM_004448.2 | Accession #: | NM_001982.2 |
| Description: | v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 | Description: | v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 |
| Kinase Species: | Human | | |
| SH2 Domain: | GRB2 | | |
| 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% CO₂ to allow the cells to attach and grow. Cells were then stimulated with a 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 |
| Control Agonist 1: | Recombinant Human Heregulinβ-1 |
| Cell Incubation Time (hours): | 24 |
| Agonist Incubation Time (minutes): | 180 |
| Agonist Incubation Temperature (°C): | RT |
| EC₅₀ for SH2 recruitment (ng/mL): | 0.59 |
| Signal:Background at agonist E_{max}: | 6.8 |



Passage Stability

This cell line has been confirmed to be stable through a minimum of 10 passages with no significant drop in assay window or change in EC₅₀.

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 | 0.25 | 92-0028 |
| AssayComplete™ Hygromycin B | 250 | 92-0029 |
| AssayComplete™ G418 | 500 | 92-0030 |

Additional Ligand Information

Control Agonist 1: Recombinant Human Heregulinβ-1

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

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