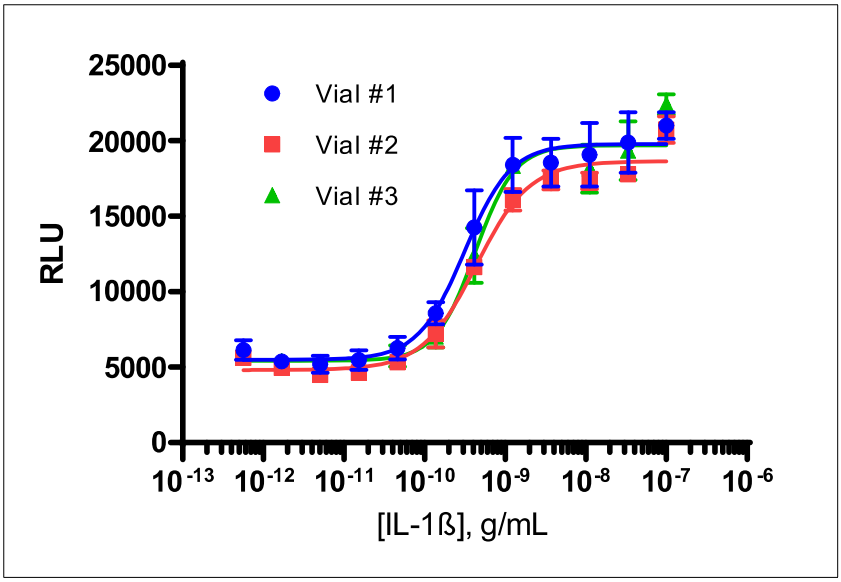
## Certificate Of Analysis

### Background

This PathHunter® Bioassay detects ligand induced dimerization of two subunits of a receptor-dimer pair. The cells have been engineered to co-express one receptor subunit fused to Enzyme Donor (ED), and a second dimer partner fused to Enzyme Acceptor (EA). Intracellular catalytic domains may have been deleted from the receptor subunits. Binding of an agonist to one receptor subunit induces it to interact with its dimer partner, forcing complementation of the two enzyme fragments. This results in the formation of a functional enzyme that hydrolyzes a substrate to generate a chemiluminescent signal.

| **Product Name** | **PathHunter® U2OS IL1R1/IL1RAP Bioassay** |
|---|---|
| Cryovial Label | U2OS IL1R1/IL1RAP Dimerization Bioassay Cells |
| Bioassay Catalog Number | 93-1032Y3 |
| Bioassay Manufactured Lot # | 22H1904 |
| Passage # @ Freezing | 4 |
| **Assay Information** | |
| Target 1 | IL1R1 |
| Target 1 Amino Acid Range | 1 - 360 |
| Target 1 Accession Number | NM_000877.3 |
| Target 1 Description | Interleukin 1 receptor, type I |
| Target 1 Tag | EA |
| Target 2 | IL1RAP |
| Target 2 Amino Acid Range | 1 - 397 |
| Target 2 Accession Number | NM_001167928.1 |
| Target 2 Description | interleukin 1 receptor accessory protein |
| Target 2 Tag | PK1 |
| Target Species | Human |
| Cell | U2OS |
| CP Reagent | AssayComplete™ Cell Plating 5 Reagent (DiscoverX, 93-0563R5A) |
| Ligand | IL-1β (DiscoverX, 92-1296) |
| Ligand Diluent | Protein Dilution Buffer |
| Detection Kit | PathHunter® Bioassay Detection Kit (DiscoverX, 93-0933) |
| Cell Number/Well | 5,000 |
| Cell Seeding Time (hours) | 24 |
| Ligand Inc Time (minutes) | 360 |
| Agonist Inc Temperature (°C) | 37 |

| Cell Density Information | | |
|---|---|---|
| Cell Number (millions) | 1.2 | |
| Fill Volume per Vial (mL) | 0.1 | |
| **Cell Viability** | | |
| Viability at Initial Thaw (%) | 92 | |
| Recovery After 48 Hours (%) | 241 | |
| **Mycoplasma and Sterility** | | |
| Mycoplasma Test | Passed | |
| Sterility Test | Passed | |
| **Functional Performance (3 manufactured vials)** | | |
| S:B Ratio | Vial 1 | 3.4 |
| | Vial 2 | 3.7 |
| | Vial 3 | 3.7 |
| $EC_{50}$ (g/mL) | Vial 1 | $0.31 \times 10^{-9}$ |
| | Vial 2 | $0.43 \times 10^{-9}$ |
| | Vial 3 | $0.42 \times 10^{-9}$ |



| Shipping and Storage Information | |
|---|---|
| Shipping Conditions | Dry Ice |
| Storage Conditions | Short term (<24 hours): -80°C; Long term (>24 hours): Vapor phase of liquid nitrogen. |
| Manufacturing Date | September 2022 |
| Expiration Date | September 2026 |

Shelf life of over 3 years has been established for DiscoverX cell lines and Assay-Ready Cells in general, when stored in the vapor phase of liquid nitrogen.

Documented by / Date: Grace Dolor

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