

## Certificate Of Analysis

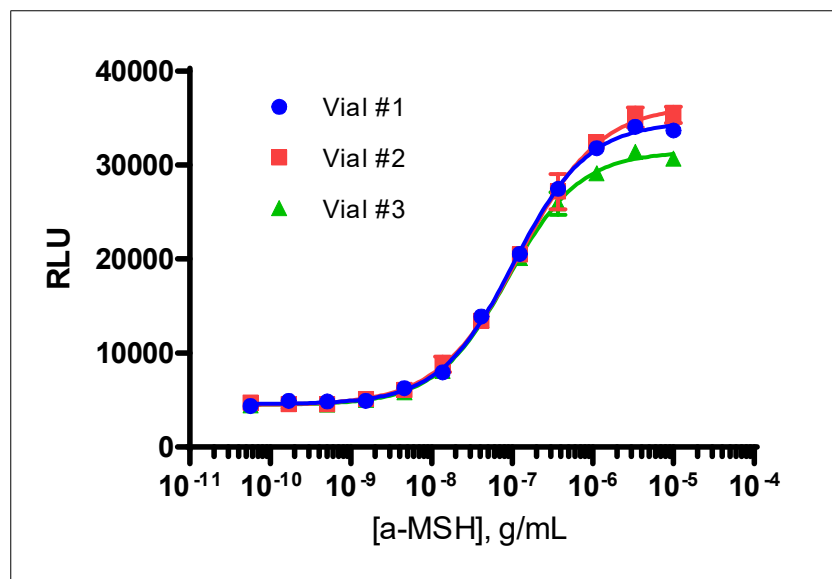
### Background

PathHunter<sup>®</sup>  $\beta$ -Arrestin GPCR Bioassay cells are engineered to co-express the ProLink<sup>™</sup> (PK) tagged GPCR and the Enzyme Acceptor (EA) tagged  $\beta$ -Arrestin. Activation of the GPCR-PK induces  $\beta$ -Arrestin-EA recruitment, forcing complementation of the two  $\beta$ -galactosidase enzyme fragments (EA and PK). The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

<b>Product Name</b>	<b>PathHunter<sup>®</sup> U2OS MC4R Bioassay Cells</b>
Cryovial Label	U2OS MC4R Beta-Arrestin Bioassay Cells
Bioassay Catalog #	93-0211Y3
Bioassay Manufactured Lot #	21K2814
Passage # @ Freezing	7

<b>Assay Information</b>	
Target 1	MC4R
Target 1 Accession Number	NM_005912.2
Target 1 Description	Melanocortin receptor 4
$\beta$ -Arrestin Isoform	$\beta$ -Arrestin-2
Target Species	Human
Cell	U2OS
CP Reagent	AssayComplete <sup>™</sup> Cell Plating 4 Reagent (DiscoverX, 93-0563R4A)
Ligand	$\alpha$ -Melanocyte stimulating hormone (DiscoverX, 92-1172)
Ligand Diluent	Protein Dilution Buffer
Detection Kit	PathHunter <sup>®</sup> Bioassay Detection Kit (DiscoverX, 93-0933)
Cell Number/Well	20,000
Cell Seeding Time (hours)	24
Ligand Inc Time (minutes)	90
Agonist Inc Temperature (°C)	37

Cell Density Information	
Cell Number (millions)	2.4
Fill Volume per Vial (mL)	0.2
Cell Viability	
Viability at Initial Thaw (%)	95
Recovery After 48 Hours (%)	148
Mycoplasma and Sterility	
Mycoplasma Test	Passed
Sterility Test	Passed
Functional Performance (3 manufactured vials)	
S:B Ratio	Vial 1 7.8
	Vial 2 7.5
	Vial 3 7.0
EC <sub>50</sub> (g/mL)	Vial 1 104.2 x 10 <sup>-9</sup>
	Vial 2 123.2 x 10 <sup>-9</sup>
	Vial 3 88.1 x 10 <sup>-9</sup>



<b>Shipping and Storage Information</b>	
Shipping Conditions	Dry Ice
Storage Conditions	Short term (<24 hours): -80°C; Long term (>24 hours): Vapor phase of liquid nitrogen.
Manufacturing Date	November 2021
Expiration Date	November 2024

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: \_\_\_\_\_

Approved by / Date: \_\_\_\_\_