

PathHunter[®] CHO-K1 EDG4 β -Arrestin Cell Line

Catalog Number: 93-0255C2 **Lot Number:** See Vial

Contents: 2 vials, 1 x 10⁶ cells per vial in 1 mL

Background

cAMP Hunter cell lines overexpress naturally coupled, wildtype GPCRs and are designed to detect increases in intracellular cAMP levels (Gs-coupled receptors) or inhibition of intracellular cAMP production (Gi-coupled receptors) in response to agonist stimulation of the receptor. These cell lines are designed to be used in conjunction with the HitHunter[®] cAMP Assay Detection Kit. cAMP Hunter cell lines expressing receptors that will couple to Gq proteins can also be used to detect induction of calcium mobilization.

Product Information

Target GPCR: EDG4

Description: Lysophosphatidic acid receptor 2

Receptor Family: Lysophospholipid (LPA)

Coupling: Gq

GPCR Species: Human

Accession Number: AY322548

Cell Type: CHO-K1

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 overnight 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 either the HitHunter cAMP Assay Detection Kit or the Calcium No Wash^{Plus} detection kit according to the recommended protocols. Please refer to page 2 for recommended assay reagents, detection reagents, and control compounds.

HitHunter cAMP Detection Assay

Cell Number/Well: 5000

Agonist Incubation Time (minutes): 180

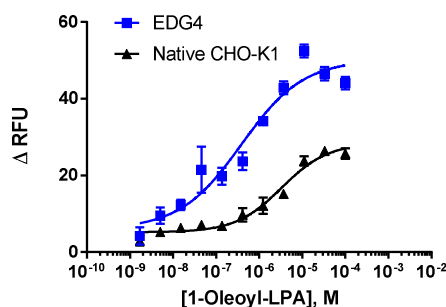
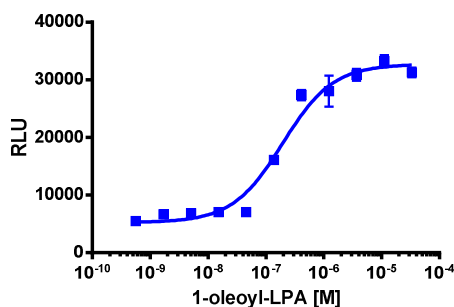
Agonist Incubation Temp. (°C): RT

Ca²⁺ Mobilization Assay

Cell Number/Well: 10000

Signal Read Time (@ 2 seconds intervals): 2 minutes

Signal Read Temperature (°C): RT



EC₅₀ for Agonist Stimulation (nM): 194

EC₅₀ for Calcium Mobilization (nM): 388

Signal:Background at agonist E_{max}: 5.7

Note: This cell line was developed and quality control tested via the HitHunter cAMP Assay Detection Kit only. Calcium mobilization assay was run independently from the HitHunter cAMP Assay Detection Kit. Calcium data is background subtracted and represented as ΔRFU (Relative Fluorescence Units).

Important! This assay requires an additional step: Please refer to Additional Protocol Information section.

Passage Stability

This cell line has been confirmed to be stable through 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	HitHunter® cAMP Assay for Small Molecules HitHunter cAMP Assay for Biologics	90-0075SM Series 90-0075LM Series
Ca ²⁺ Detection Reagent	Calcium No Wash ^{PLUS}	90-0091
Cell Culture	AssayComplete™ Cell Culture Kit-108	92-3108G
Cell Plating	AssayComplete™ Cell Plating 19 Reagent	93-0563R19A
Cell Detachment	AssayComplete™ Cell Detachment Reagent	92-0009
Cell Thawing	AssayComplete™ Thawing Reagent T2	92-4102TR
Cell Freezing	AssayComplete™ Freezing Reagent F2	92-5102FR

*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	300	92-0029
AssayComplete™ G418	800	92-0030

Additional Ligand Information

Control Agonist: 1-Oleoyl-LPA

Vendor: DiscoverX® (Catalog No. 92-1041)

Additional Protocol Information

If running a Calcium flux assay with these cells, it is important that the buffer (e.g. HBSS/20mM HEPES) used as the diluent for the agonist dose-response dilution curve (or EC80 challenge) contains 0.1% BSA.

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. For additional information, please visit discoverx.com.

Limited Use License Agreement

These products may be covered by issued US and/or foreign patents, patent application and subject to Limited Use Label License.

Please visit discoverx.com/license for a list of products that are governed by limited use label license terms and relevant patent and trademark information.