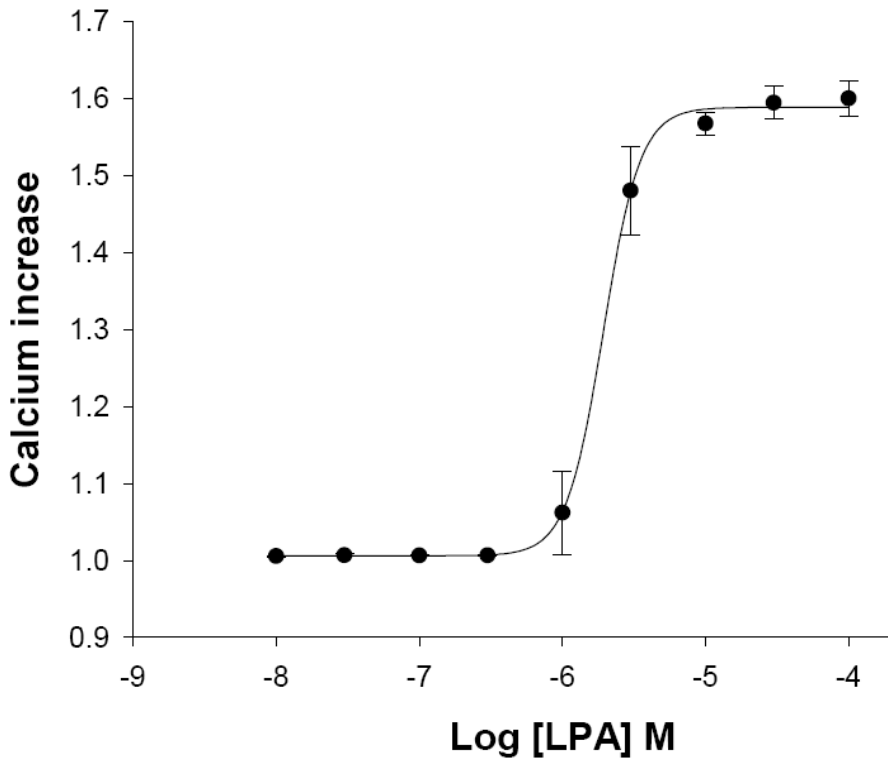
REF: P30156

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- LYSOPHOSPHATIDIC ACID RECEPTOR 3 (LPA-3) CELL LINE -



Product name: LPA_3 (EDG7) /U2OS cell line

Ec_{50} LPA: 1.93×10^{-6} M

Z´: 0.88+/- 0.02

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REF: P30156

- LYSOPHOSPHATIDIC ACID RECEPTOR 3 (LPA-3) U2OS CELL LINE -

Product Name:	LPA_3 (EDG7)/U2OS
Official Full Name:	Lysophosphatidic acid receptor 3
DNA Accession Number:	GenBank: AY322547
Host Cell:	U2OS
Format:	2 cryopreserved vials
Resistance:	G418
References:	

- ***P30156:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30156-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTseeker LPA_3 contains U2OS cells stably expressing human Lysophosphatidic acid receptor 3 (LPA_3) with no tag.

Innoprot's HiTSeeker LPA_3 cell line has been designed to assay compounds or analyze their capability to modulate Lysophosphatidic acid receptor 3. When the agonist binds to LPA_3 a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Calcium).

This cell line has been validated measuring calcium increase in the cytosol. The high reproducibility of this assay allows monitoring LPA_3 activation process in High Throughput Screening.

About LPA_3

The **lysophospholipid receptor** (LPL-R) group (also referred to as EDG, **endothelial differentiation gene**)are members of the G protein-coupled receptor family of integral membrane proteins that are important for lipid signaling.

LPA receptors are implicated in several biologic roles, such as platelet aggregation, proliferation, chemotaxis, smooth muscle contraction, and tumour cell invasion.

LPA_3 receptor is expressed in testis, prostate, heart, lung and brain.

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Assay Characterization

Our expression plasmid contains the coding sequence of human LPA_3 protein. Our plasmid was transfected in U2OS cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

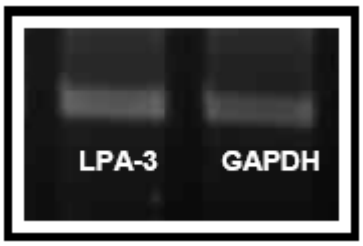

Fig.1. LPA_3 and GAPDH housekeeping gene RT-PCR.

Cells were incubated with Fura2-AM and treated with increasing LPA concentrations.

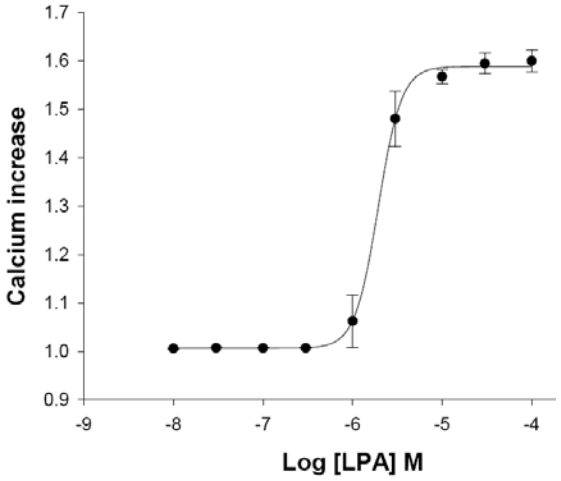

Fig.2. LPA_3 dose response in calcium assay. Cells were treated with **LPA** concentrations ranging from 0 to 100 µM, n=5. The EC50 for **LPA** was ~**$1.93x10^{-6}$M**. The calcium assay was validated with a Z´= 0.88+/- 0.02 for High Throughput Screening.

Validation of LPA_3 cell line

Calcium assay (Ec50 = 1.93×10^{-6}M)

A typical fluorescent calcium assay was performed using Fura-2/AM ratiometric. Calcium increase inside the cell was measured using the ratio of the fluorescence from Fura2 bound and not bound to the ion. Image acquisition was performed using a "BD Pathway 855" High-Content Bioimager from BD Biosciences.

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