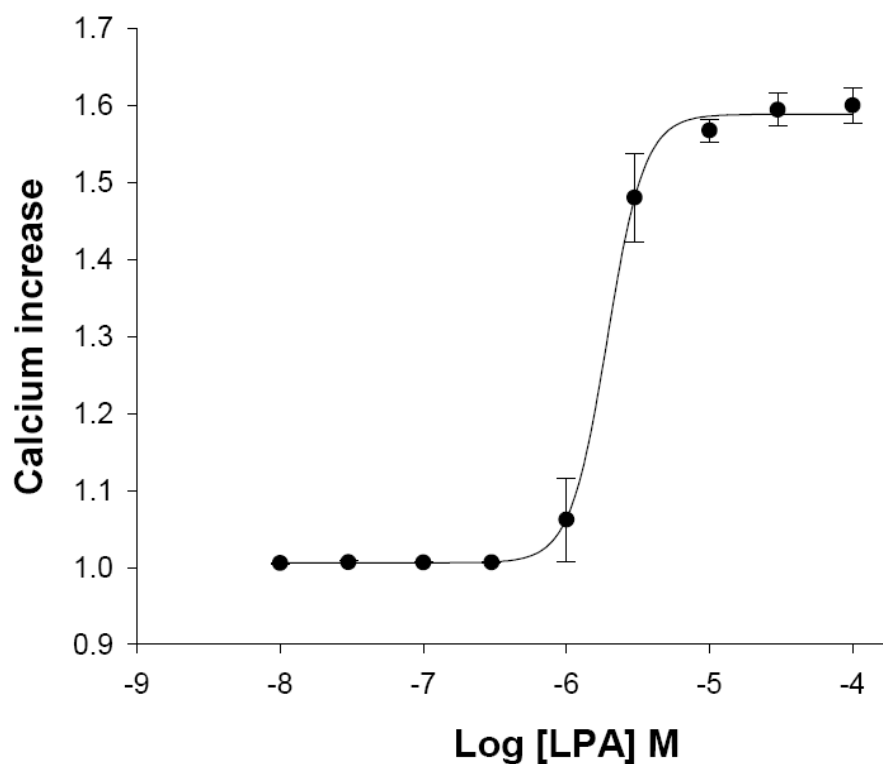


HiTSeeker CELL LINES (LABEL-FREE GPCRS)
- LYSOPHOSPHATIDIC ACID RECEPTOR 3 (LPA-3) CELL LINE -





Product name: LPA₃ (EDG7) /U2OS cell line

Ec₅₀ LPA: 1.93 x 10⁻⁶ M

Z': 0.88+/- 0.02

- 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 LPA₃ contains U2OS cells stably expressing human Lysophosphatidic acid receptor 3 (LPA₃) with no tag.

Innoprot's HiTSeeker LPA₃ cell line has been designed to assay compounds or analyze their capability to modulate Lysophosphatidic acid receptor 3. When the agonist binds to LPA₃ 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 LPA₃ activation process in High Throughput Screening.

About LPA₃

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.

LPA₃ receptor is expressed in testis, prostate, heart, lung and brain.

Assay Characterization

Our expression plasmid contains the coding sequence of human LPA₃ 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).

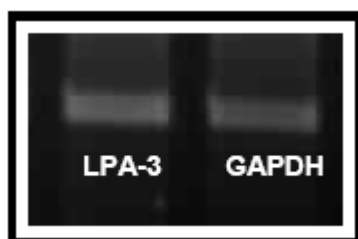


Fig.1. LPA₃ and GAPDH housekeeping gene RT-PCR.

Validation of LPA₃ cell line

Calcium assay (EC₅₀ = 1.93 x 10⁻⁶M)

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.

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

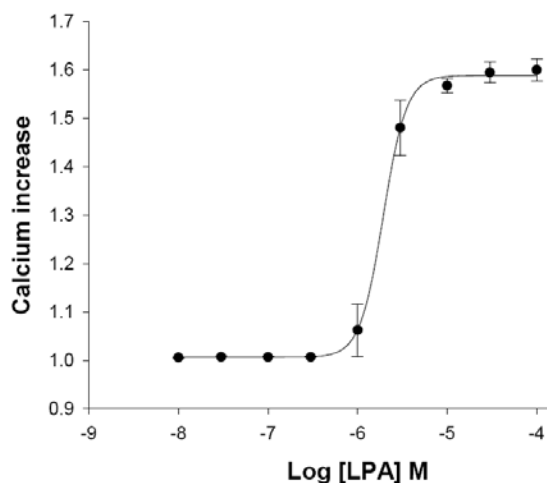


Fig.2. LPA₃ dose response in calcium assay.

Cells were treated with LPA concentrations ranging from 0 to 100 μM, n=5. The EC₅₀ for LPA was 1.93×10^{-6} M. The calcium assay was validated with a Z' = 0.88 +/- 0.02 for High Throughput Screening.