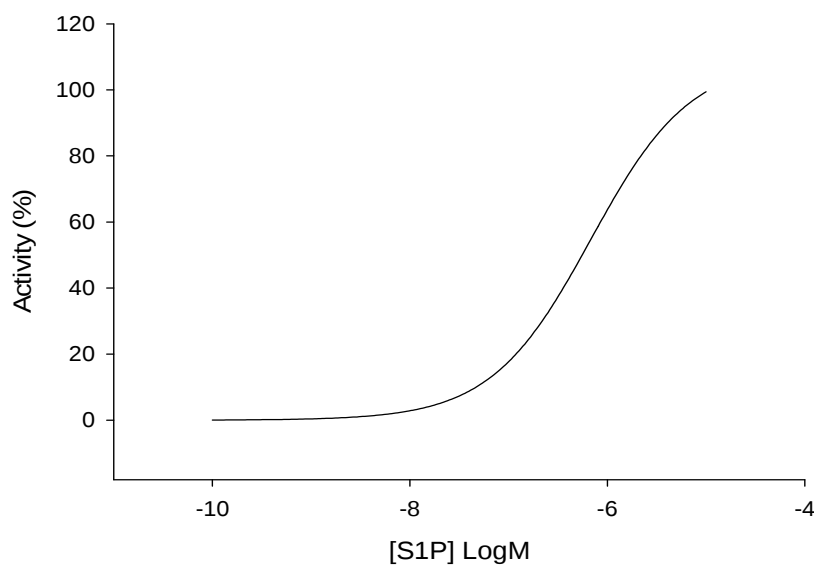
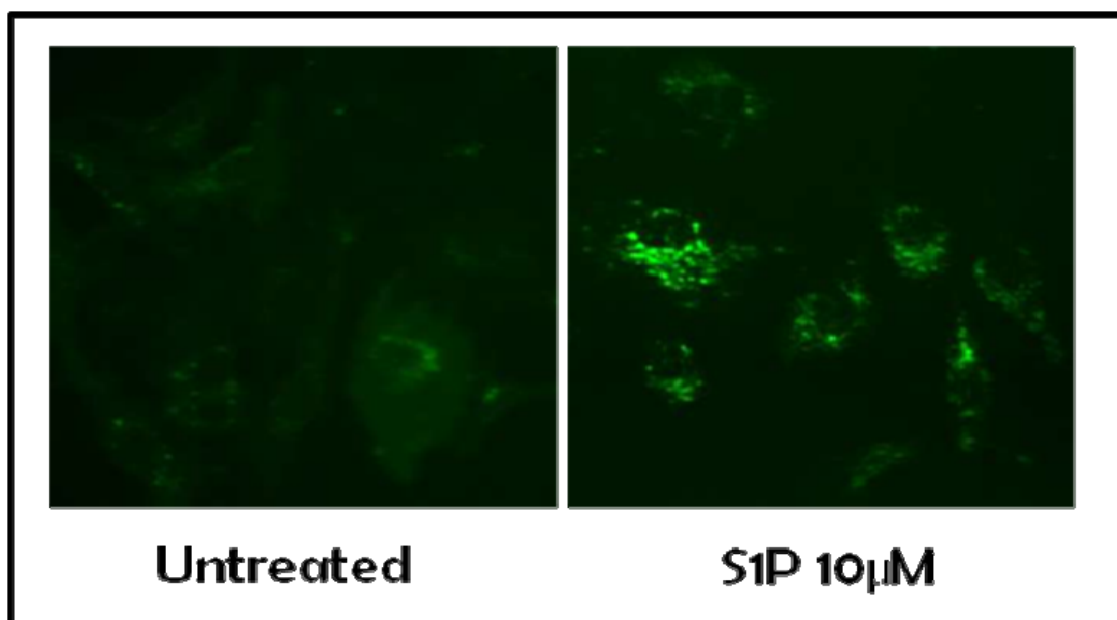


RECEPTOR INTERNALIZATION ASSAYS

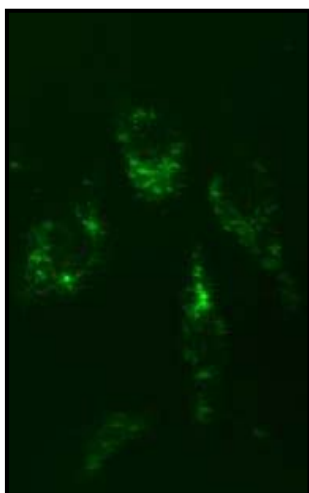
- FLUORESCENT SPHINGOSINE-1-PHOSPHATE RECEPTOR 1 CELL LINE -



Product name: S1P₁R-tGFP / HepG2 Cell Line

EC₅₀ S1P: 6.9 x 10⁻⁷ M

Z': 0.71+/- 0.02



Product Name: S1P₁R-tGFP/HepG2

Receptor Name: Human Sphingosine-1-phosphate receptor 1

DNA Accession Number: GenBank NM_001400

Host Cell: HepG2

Format: Cryopreserved vials

References:

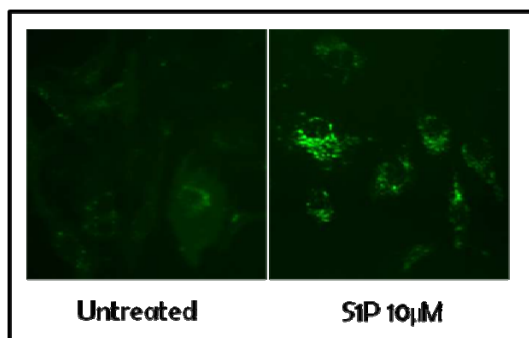
P30226: 2 vials of 3 x 10⁶ proliferative cells

P30226-DA: 1 vial of 2 x 10⁶ division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

Human Sphingosine-1-phosphate receptor 1-tGFP/HepG2 contains HepG2 cells stably expressing human Sphingosine-1-phosphate receptor 1 (S1P₁R) tagged in the C-terminus with tGFP. Innoprot S1P₁R redistribution Assay kit has been designed to assay compounds or analyze stimuli for their ability to modulate S1P₁ receptor activation and the following redistribution process inside the cells.



This highly reproducible assay allows monitoring S1P₁ receptor activation and redistribution process in High Content Analysis and fluorescence microscope applications.

About S1P₁ Receptor

This gene encodes S1P₁ receptor which is a member of the G protein-coupled receptor superfamily (S1P₁₋₅) originally known as EDG receptors. Through the interaction with this receptor family, sphingosine-1-phosphate stimulates diverse cellular responses, including cytoskeletal changes, proliferation and migration. The S1P₁R is widely expressed including on endothelial cells. The physiological effects of S1P₁R have been described in immune and vascular systems

Assay Characterization

Our expression plasmid containing the coding sequence of human S1P₁R tagged in the C-terminal with tGFP protein. Our plasmid was transfected in HepG2 cells. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

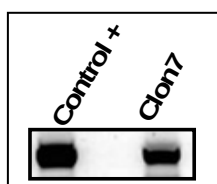


Fig.1. Clones S1P₁R mRNA expression.

Activation and Internalization assay for S1P₁R-tGFP

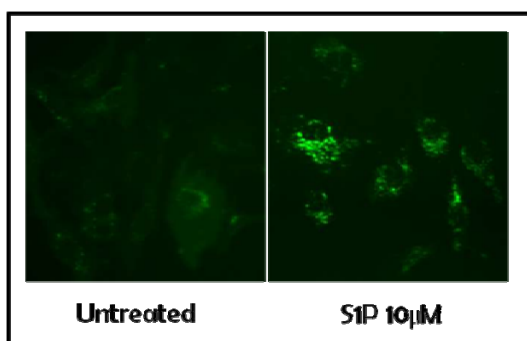


Fig.2. Redistribution of S1P₁R stimulated with isoproterenol. Cells were treated with 10 μM S1P₁ for h. Activation and redistribution processes were detected and analyzed using “BD Pathway 855” High-Content Bioimager from BD Biosciences.

Assay Details

HepG2 stably expressing human S1P₁R tagged in the C-terminus with tGFP were stimulated with different concentrations of S1P₁ agonist during 1 hour. After that, the nucleus was stained with DAPI and S1P₁R fluorescence redistribution was detected by fluorescence using image analysis algorithms. When cells were treated with the agonist, the human S1P₁R was internalized in high intensity vesicles. The activity was calculated as an increment of intensity of these vesicles.

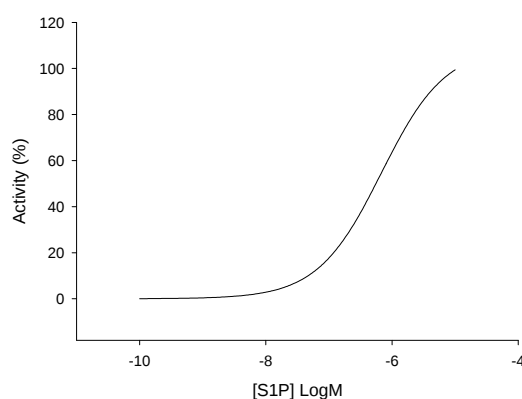


Fig.3. S1P₁ concentrations response in the S1P₁R redistribution assay. Cells were treated with 6 log dilution series (n=8). The EC₅₀ for the S1P was ~ 0.69 μM after a treatment of 1h with agonist. Cells were fixed and the nucleus were stained with DAPI. % Activity was calculated relative to positive (10 uM). The internalization assay was validated with an average of Z' = 0.71 ± 0.02 for High Content Screening.