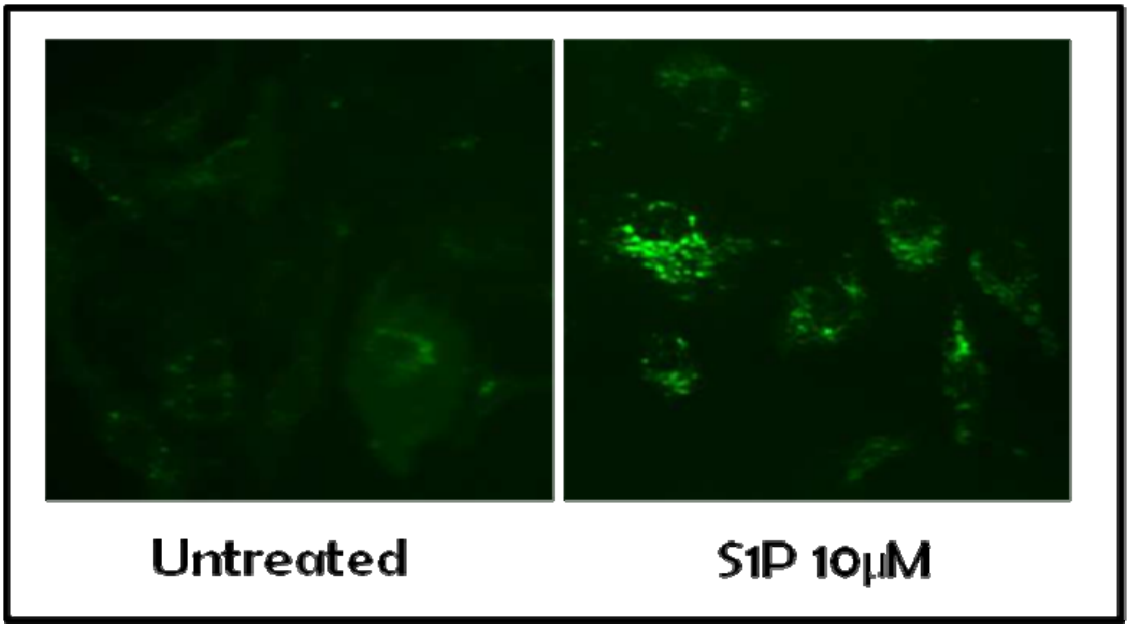
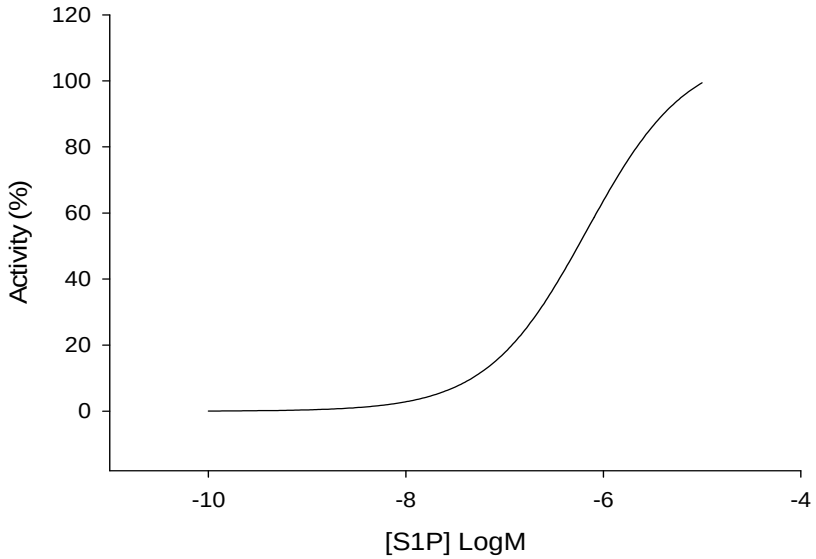
REF: P30226

RECEPTOR INTERNALIZATION ASSAYS

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





Product name: S1P$_1$R-tGFP / HepG2 Cell Line

Ec$_{50}$ S1P: 6.9 x 10^{-7} M

Z´: 0.71+/- 0.02

VAT No. ESB95481909

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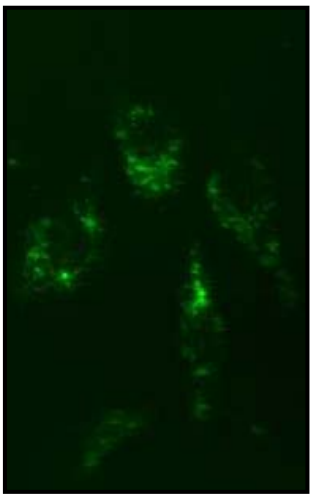


Product Name: $S1P_1R$-tGFP/HepG2

Receptor Name: Human Sphingosine-1-phosphate receptor 1

DNA Accesion Number: GenBank NM_001400

Host Cell: HepG2

Format: Cryopreserved vials

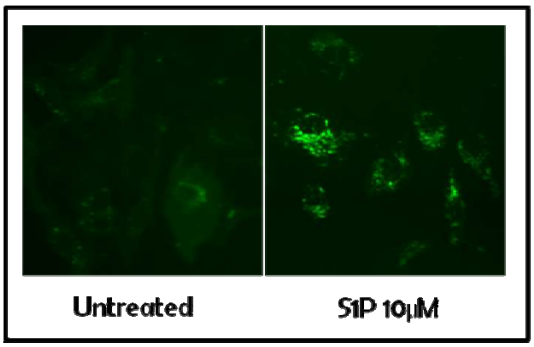
References:

- ***P30226:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30226-DA***: 1 vial of 2 x 10^6 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 ($S1P_1R$) tagged in the C-terminus with tGFP. Innoprot $S1P_1R$ redistribution Assay kit has been designed to assay compounds or analyze stimuli for their ability to modulate $S1P_1$ receptor activation and the following redistribution process inside the cells.



This highly reproducible assay allows monitoring $S1P_1$ receptor activation and redistribution process in High Content Analysis and fluorescence microscope applications.

About $S1P_1$ Receptor

This gene encodes $S1P_1$ receptor which is a member of the G protein-coupled receptor superfamily ($S1P_{1-5}$) 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 $S1P_1R$ is widely expressed including on endothelial cells. The physiological effects of $S1P_1R$ have been described in immune and vascular systems

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Assay Characterization

Our expression plasmid containing the coding sequence of human $S1P_1R$ 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).

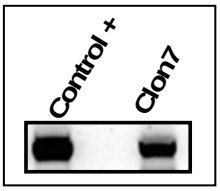

Fig.1. Clones $S1P_1R$ mRNA expression.

Activation and Internalization assay for $S1P_1R$ -tGFP

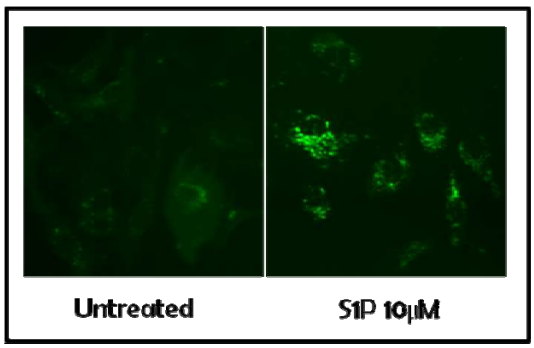

Fig.2. Redistribution of $S1P_1R$ stimulated with isoproterenol. Cells were treated with 10 µM $S1P_1$ for h. Activation and redistribution processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

HepG2 stably expressing human $S1P_1R$ tagged in the C-terminus with tGFP were stimulated with different concentrations of $S1P_1$ agonist during 1 hour. After that, the nucleus was stained with DAPI and $S1P_1R$ fluorescence redistribution was detected by fluorescence using image analysis algorithms. When cells were treated with the agonist, the human $S1P_1R$ was internalized in high intensity vesicles. The activity was calculated as an increment of intensity of these vesicles.

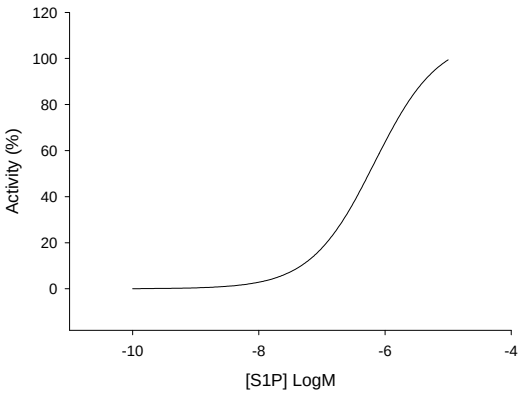

Fig.3. $S1P_1$ concentrations response in the $S1P_1R$ redistribution assay. Cells were treated with 6 log dilution series (n=8). The Ec50 for the S1P was ~ 0.69 µM after a treatment of 1h with agonist. Cells were fixed and the nucleus were stained with DAPI. % Activity was calculated relative to positive (10 uM). The internalization assay was validated with an average of Z´=0.71+/- 0.02 for High Content Screening.

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