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Sphingosine-1-Phosphate Receptor 5 (S1PR5) ACTOne™ Stable Cell Line

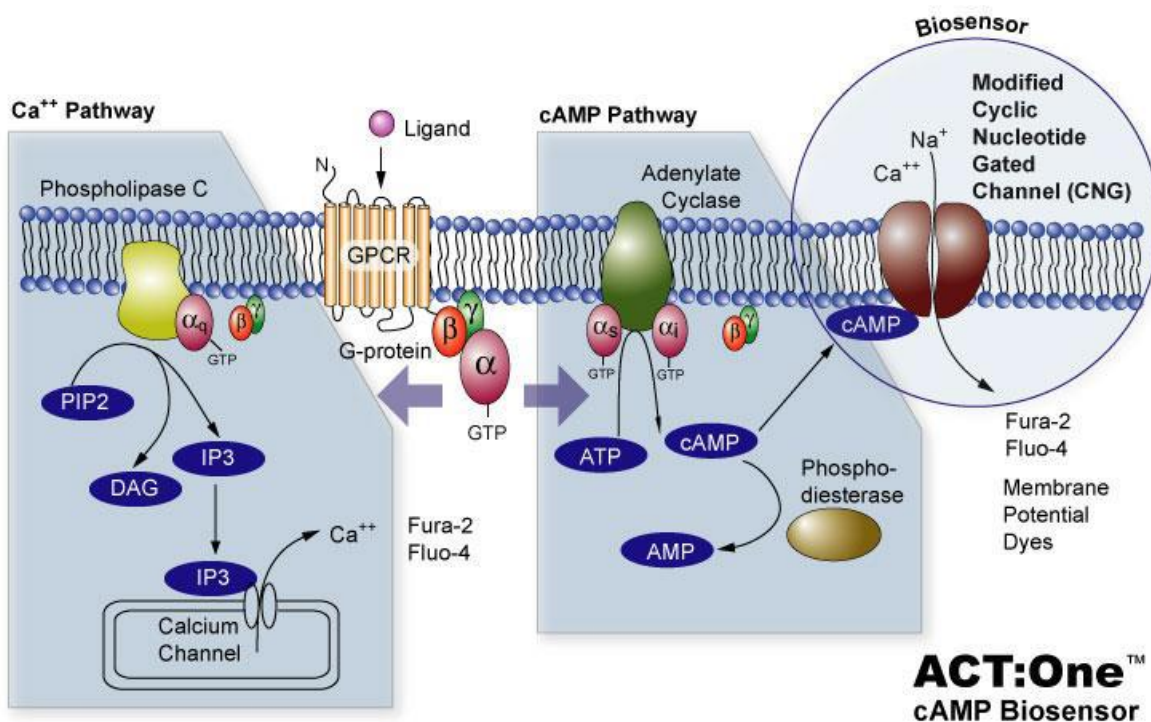
CATALOG NUMBER: CL-01-S1PR5

Introduction

S1PR5 is a G-protein-coupled receptor which binds the bioactive signaling molecule sphingosine 1-phosphate (S1P). S1PR5 belongs to a sphingosine-1-phosphate receptor subfamily comprising five members (S1PR5-5). They are expressed in a wide variety of tissues, with each subtype exhibiting a different cell specificity, although they are found at their highest density on leukocytes. S1PR5 may play a regulatory role in the transformation of radial glial cells into astrocytes and may affect proliferative activity of these cells.

Description

Human S1PR5 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human S1PR5. HEK-293 CNG cells express a modified CNG (Cyclic Nucleotide Gated) channel that opens in response to elevated intracellular cAMP levels and consequently result in ion flux (often detectable by calcium-responsive dye, [Cat# CA-C155](#)) and cell membrane depolarization which can be easily measured with fluorescent Membrane Potential Dye ([Cat# CA-M165](#)). The assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FDSS, FLIPR, or a fluorescence microplate reader.



Parental Cells

HEK-293 CNG cells (originally developed by BD Biosciences by introducing CNG in HEK-293 cells) (Cat# CL-03-PC20)

Gene/Enzyme Introduced

S1PR5 (Genbank Accession No. NP_001159687.1)

Applications

- cAMP dependent human S1PR5 receptor cell based assay

- cell based high-throughput screening of human S1PR5 receptor agonists/antagonists

Functional Test

- this cell line has been tested positive for S1PR5 receptor specific response
- surviving rate: More than 2.5 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of mycoplasma contamination.

Content

- Stable S1PR5 receptor cells: 1 mL (1 x 10⁶ cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 µg/ml G418, 1 µg/ml Puromycin
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Data Example

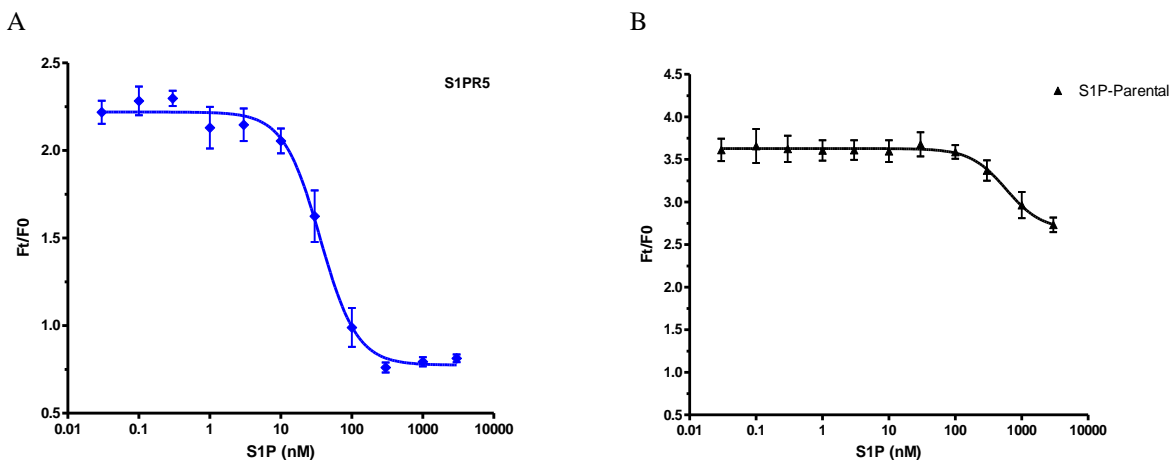


Figure 1. Response of ACTOne™ S1PR5 cell line & parental cell line to S1P

ACTOne™ S1PR5 cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hours of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of S1P. Ratios of the two readings (F/F₀) are plotted in the figure.

- Dose response curve of S1P in ACTOne™ S1PR5 cell line. EC₅₀ = 36 nM in the presence of PDE inhibitor Ro 20-1724 and β-adrenoceptor agonist isoproterenol.**
- Parental cells do not respond to S1P.**

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