

## Sphingosine-1-phosphate Receptor 1 (S1PR1) ACTOne™ Stable Cell Line

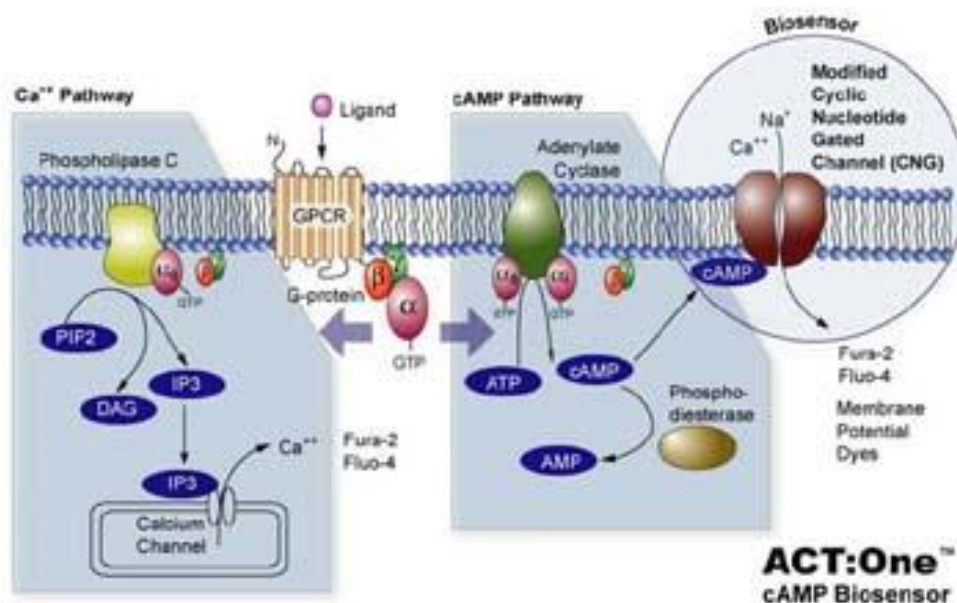
CATALOG NUMBER: CL-11-S1PR1

### Introduction

Sphingosine-1-phosphate receptor 1 (S1PR1), also known as endothelial differentiation gene 1 (EDG1) is a G-protein-coupled receptor which binds the bioactive signaling molecule sphingosine 1-phosphate (S1P). S1PR1 belongs to a sphingosine-1-phosphate receptor subfamily comprising five members (S1PR1-5). S1PR1 was originally identified as an abundant transcript in endothelial cells and it has an important role in regulating endothelial cell cytoskeletal structure, migration, capillary-like network formation and vascular maturation. In addition, S1PR1 signaling is important in the regulation of lymphocyte maturation, migration and trafficking.

### Description

Human S1PR1 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human S1PR1. 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 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

S1PR1 (Genbank Accession No. NP\_001391.2)

### Applications

- cAMP dependent assay for Gi-coupled human S1PR1
- cell based high-throughput screening of human S1PR1 inhibitors

### Functional Test

- this cell line has been tested positive for S1PR1 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 cells: 1 mL (1 x 10<sup>6</sup> cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

### Growth Properties

Adherent

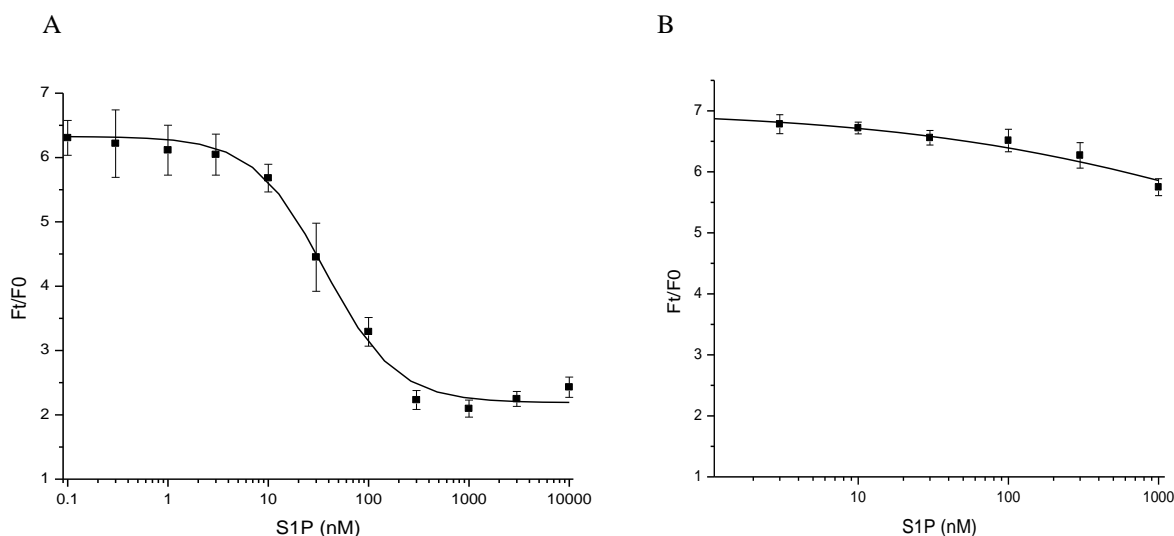
### Cell Culture Medium

- 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 Analysis



#### Figure 1. Response of ACTOne™ S1PR1 cell line & parental cell line to S1P.

ACTOne™ S1PR1 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/F<sub>0</sub>) are plotted in the figure..

- Dose response curve of S1P in ACTOne™ S1PR1 cell line. EC<sub>50</sub> = 36 nM in the presence of PDE inhibitor Ro 20-1724 and β-adrenoceptor agonist isoproterenol.
- Parental cells do not respond to S1P.

### Notice to Purchaser

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