

ACTOne™ Human Sphingosine-1-Phosphate Receptor 3 (S1PR3) Stable Cell Line

Catalog Number: CL-11-S1PR3

Product Type: Stable Cell Line

Cell Line Name: ACTOne™ S1PR3

Host Cell Line: HEK-293 CNG

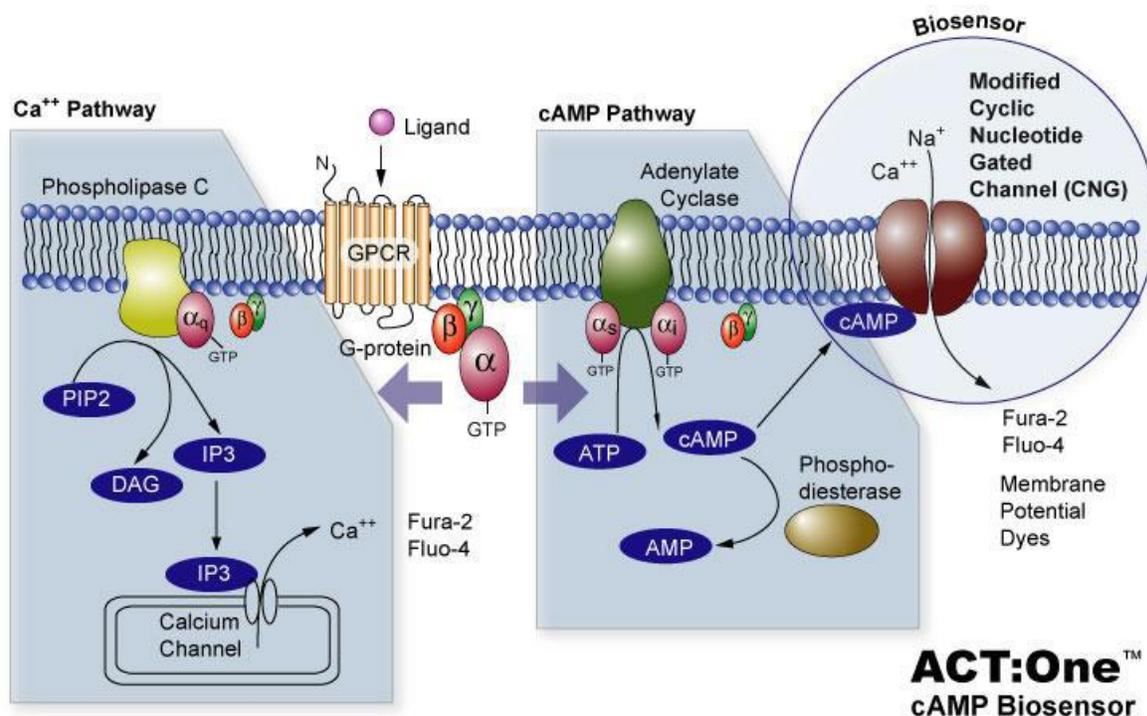
Target Receptor: Human Sphingosine-1-Phosphate Receptor 3 (S1PR3)

GenBank Accession: NP_005217.2

Product Description

The ACTOne™ S1PR3 stable cell line is a HEK-293 CNG derivative engineered to express recombinant human sphingosine-1-phosphate receptor 3 (S1PR3). These cells contain a modified cyclic nucleotide-gated (CNG) channel that opens in response to intracellular cAMP elevation, triggering ion flux and membrane depolarization. This response is quantifiable using fluorescent membrane potential dye (Cat# CA-M165) or calcium-responsive dye (Cat# CA-C155).

The assay supports both endpoint and kinetic measurements of cAMP signaling and is compatible with FLIPR and fluorescence microplate readers



Applications

- Calcium flux assays for Gq-coupled S1PR3
- High-throughput screening of S1PR3 agonists and antagonists
- GPCR pathway analysis

Functional Validation

- **S1PR3-specific response:** Confirmed
- **Post-thaw viability:** >2.5 million cells/vial on Day 2
- **Receptor activity stability:** Maintained over 10 weeks of continuous passage
- **Mycoplasma status:** Negative (tested lot-specific)

Product Format

- **Volume:** 1 mL
- **Cell Density:** 1×10^6 cells/mL
- **Cryopreservation Medium:** 70% DMEM, 20% FBS, 10% DMSO

Growth Characteristics

- **Culture Type:** Adherent
- **Growth Medium:** DMEM + 10% FBS, supplemented with 250 μ g/mL G418 and 1 μ g/mL Puromycin
- **Freezing Medium:** 10% DMSO in complete growth medium

Subculturing Protocol

1. Thaw Cryovial in 37 °C water bath (1-2 min).
2. Decontaminate vial with 70% ethanol.
3. Transfer contents to a 75 cm² flask with 20 ml of complete DMEM growth medium.
4. Replace medium after 24 hours.
5. Remove and discard culture medium next day and then add fresh DMEM complete medium.
6. Monitor daily; passage at 90% confluence (1:3 split).
7. Use 0.25% trypsin-0.53 mM EDTA for detachment (15-20 min at 37°C).
8. Centrifuge at 250 x g for 5 to 10 min.
9. Resuspend and seed at 4-6 x 10⁴ viable cells/cm².
10. Incubate at 37°C, 5% CO₂.

Freezing & Storage Instructions

1. Trypsinize cells and resuspend in freezing medium at 2.5 x 10⁶ cells/mL.
2. Aliquot 1 mL per cryovial.
3. Freeze overnight at -80°C in a cryo container.
4. Transfer to vapor-phase liquid nitrogen (\leq -130°C) for long-term storage.



Data Summary

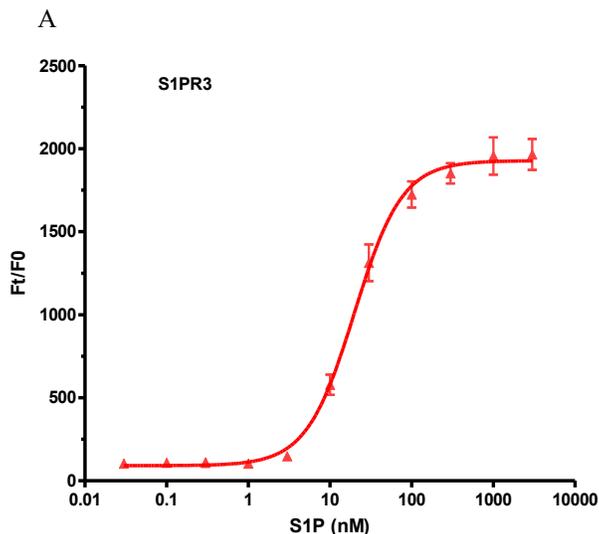


Figure 1. Response of ACTOne™ S1PR3 cell line

ACTOne™ S1PR3 cells and parental HEK-293 CNG cells 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 (Elite™ Non-Wash Calcium Dye, Cat# CA-C115a). After 1 hour of incubation at 37 °C, the plate was read on Hmamastu FDSS 7000 with online addition of the ligand. Fluorescent intensity was used to plot the figure.

- A. Dose response curve of S1P in ACTOne™ S1PR3 cell line. EC50 = 20 nM**
- B. Parental cells do not respond to S1P (data not shown)**

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