

Product Information



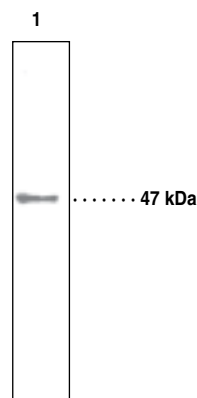
S1P₃ Polyclonal Antibody

Item No. 10006373 • Lot No. XXXX

- Synonyms:** EDG-3, S1PR3, Sphingosine-1-phosphate Receptor 3
- Supplied as:** *lot specific* µg peptide affinity-purified IgG in *lot specific* µl TBS, pH 7.4, containing 50% glycerol, 0.1% BSA, and 0.02% sodium azide
- Host:** Rabbit
- Antigen:** Human S1P₃ amino acids 12-25; the antigen alignment with other known sequences is as follows:
Human VRGNETLREHYQYV
Mouse and Rat V I G N d T L R E H Y d Y V
- Cross-reactivity:** (+) Human, mouse, and rat S1P₃; other species not tested
- Stability:** ≥1 year at -20°C
- Applications:** For detection of S1P₃ by western blot (WB) and immunocytochemistry (ICC). Recommended starting dilution for WB is 1:200 (*lot specific* µg/ml) and for immunocytochemistry. Other applications were not attempted and therefore optimal working dilutions should be determined empirically.

Sphingosine-1-phosphate (S1P) exerts its activity by binding to five distinct G-protein-coupled receptors, S1P₁/EDG-1, S1P₂/EDG-5, S1P₃/EDG-3, S1P₄/EDG-6, and S1P₅/EDG-8.^{1,2} S1P₃ couples to G_{i/o}-ERK, G_q-PLC, and G_{12/13}-Rho axes to mediate S1P-induced cell proliferation, survival, migration, and related signaling events.¹⁻³ S1P₃ is widely expressed in various tissues, suggesting diverse physiological functions of this receptor.⁴ Human and mouse S1P₃ have 378 amino acids with an estimated molecular weight of 42 kDa. Glycosylation at the N-terminal extracellular domain may cause the protein to migrate at different positions in SDS-PAGE.

Cayman's S1P₃ polyclonal antibody detects the receptor at 47 kDa using samples from human cerebral cortex. Liver and heart tissues display 2-3 bands between 40-50 kDa, possibly due to different degree of glycosylation.



Lane 1: Human cerebral cortex membrane (30 µg)

Laboratory Procedures

Immunocytochemistry (ICC)

1. Grow cells in 12 or 24 well plates until confluence.
2. Wash cells 3 times in TBS, pH 7.4, 5 minutes each.
3. Fix the cells with 1% formaldehyde in TBS, pH 7.4, for 10 minutes.
4. Wash cells 3 times in TBS, pH 7.4, containing 0.1% Triton X-100 (TBSTX) for 10 minutes each.
5. Incubate cells with 10% normal serum (same species as secondary antibody host) in TBSTX, for 30 minutes.
6. Rinse cells 3 times with TBSTX, pH 7.4, 5 minutes each.
7. Incubate cells with 4 µg/ml S1P₃ polyclonal antibody (recommended starting dilution; optimal dilution to be determined by end user) for 1 hour at room temperature.
8. Wash cells 3 times in TBSTX, pH 7.4, 10 minutes each.
9. Incubate cells in the dark for 1 hour with a fluorochrome-conjugated secondary antibody in TBSTX, pH 7.4, using a dilution as recommended by provider.
10. Wash cells 3 times in TBSTX, pH 7.4, 10 minutes each.
12. The stained cells are now ready to be examined under a microscope with appropriate filter. Store the plate in the dark, at 4°C, for later analysis if necessary.

WARNING: THIS PRODUCT IS FOR LABORATORY RESEARCH ONLY. NOT FOR ADMINISTRATION TO HUMANS. NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

MATERIAL SAFETY DATA

This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Material Safety Data Sheet, which has been sent via email to your institution.

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References

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3. Kluk, M.J. and Hla, T. Signaling of sphingosine-1-phosphate *via* the S1P/EDG-family of G-protein-coupled receptors. *Biochim. Biophys. Acta* **1582**, 72-80 (2002).
4. Takuwa, Y. Subtype-specific differential regulation of Rho family G proteins and cell migration by the Edg family sphingosine-1-phosphate receptors. *Biochem. Biophys. Acta* **1582**, 112-120 (2002).

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