

Product Information

S1P₅ C-Terminal Blocking Peptide

Catalog Number **E8278**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

Synonym: EDG-8 (Endothelial Cell Differentiation Gene 8) C-Terminal Blocking Peptide

Product Description

The S1P₅ C-terminal blocking peptide is a synthetic peptide (~2.5 kDa) derived from C-terminal domain of the full length S1P₅ (EDG-8, Endothelial Cell Differentiation Gene 8) receptor (~42 kDa).

The peptide is used to immunize rabbits and raise Anti-S1P₅ C-terminal antibody (Catalog Number E7528). It is also used as a blocking peptide in immunoblotting applications with the Anti-S1P₅ C-terminal antibody. The preincubation of the Anti-EDG-8 with blocking peptide neutralizes the antibody and renders it inactive.

The lysosphingolipid sphingosine-1-phosphate (S1P) and the structurally related lipid lysophosphatidic acid (LPA) elicit a wide spectrum of biological responses in a variety of cell types, including calcium mobilization, mitogenesis, cell-shape changes, migration, and contraction. Studies have identified the existence of the G protein-coupled heptahelical receptor subfamily (Endothelial Cell Differentiation Genes), which consists of the two receptor subgroups specific for S1P and LPA, respectively. The S1P receptor subgroup comprises five members, i.e., EDG-1, 3, 5/AGR16, 6, and 8, with considerable amino acid similarity among them. The LPA subgroup includes EDG-2, 4, and 7.^{1,2}

The EDG-8 receptor (400 amino acids) shares 42–49% amino acid identity with the other human S1P receptors EDG-1, 3, and 5. Expression of EDG-8 leads to high-affinity binding for labeled S1P. The EDG-8 transcript was widely expressed in rat tissues, with strongest expression in astrocytes in rat brain, spleen, and white matter tracts of the brain.^{3–5} In Chinese hamster ovary cells heterologously expressing EDG-8, S1P inhibited forskolin-induced cAMP accumulation and activated c-Jun NH₂-terminal kinase. Together the data demonstrate EDG-8 is a high affinity S1P receptor that couples to G(i/o)alpha proteins and is expressed predominantly in rat brain.

Reagent

Supplied in a solution of phosphate buffered saline.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at $-20\text{ }^{\circ}\text{C}$. Upon initial thawing, for extended storage, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing of the peptide. The product remains active for at least 12 months when stored appropriately.

Procedure

Preincubate undiluted Anti-S1P₅ C-terminal antibody (Catalog Number E7528) with S1P₅ C-terminal blocking peptide for 20 minutes at $37\text{ }^{\circ}\text{C}$. Use at least 50-fold stoichiometric excess of peptide. After the incubation, dilute antibody according to protocol for Catalog Number E7528 and perform immunoblotting using RH7777 cells transfected with full length EDG-8 receptor. Preincubation of the antibody with this peptide abrogated EDG-8 detection, while preincubation with a non-specific peptide had no effect.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Kupperman, E. et al., A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. *Nature*, **406**, 192-195 (2000).
2. Takuwa, Y. et al., Subtype-specific, differential activities of the EDG family receptor sphingosine-1-phosphate, a novel lysophospholipid mediator. *Mol. Cell Endocrinol.*, **177**, 3-11 (2001).
3. Fitzgerald, L.R. et al., Identification of an EDG7 variant, HOFNH30, a G-protein-coupled receptor for lysophosphatidic acid. *Biochem. Biophys. Res. Commun.*, **273**, 805-810 (2000).
4. Lee, M.J. et al., Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science*, **279**, 1552-1555 (1998).
5. Bandoh, K. et al., Molecular cloning and characterization of a novel human G-protein coupled receptor, EDG7, for lysophosphatidic acid. *J. Biol. Chem.*, **274**, 27776-27785 (1999).

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