



EDG5 (S1P₂) Functional Receptor Membrane Preparation

Functional Endothelial Cell Differentiation Gene Receptor-5 (S1P₂) Membrane Preparation

BACKGROUND

EDG-5 belongs to a family of G-protein coupled receptors whose ligands are lysophospholipids. The ligand for EDG-5 is sphingosine-1-phosphate. There are 8 known members of the EDG receptor family and they are implicated in mediating growth related effects such as induction of cellular proliferation, alterations in differentiation and survival and suppression of apoptosis. They also evoke cellular effector functions that are dependent on cytoskeletal responses such as contraction, secretion, adhesion and chemotaxis. EDG receptors are developmentally regulated and differ in tissue distribution. They couple to multiple types of G proteins to signal through ras and MAP kinase, rho, phospholipase C and several protein tyrosine kinases. EDG-5 is expressed in cardiovascular, leukocyte-containing and other tissues.

PROTOCOL

1. Add 10 to 15 μ l of membrane preparation (lot specific, see vial label for exact volume) to 1 ml of binding buffer, with vortexing of membrane preparation stock every minute to avoid settling of the suspension. (Binding buffer formulation: 50 mM HEPES (pH 7.5), 100 mM NaCl, 1 mM MgCl₂, 10 μ M GDP, 2 mM dithiothreitol, 0.1 nM [³⁵S]GTP S).
2. Add varying quantities of sphingosine-1-phosphate up to a maximum concentration of 1-2 μ M to the binding buffer as quickly as possible and mix the samples.
3. Incubate samples for 30 min at 30°C.
4. Wet Whatman GF/B glass filters and apply samples.
5. Wash samples by rapid vacuum filtration or centrifugation three times with wash buffer at 2 ml of wash buffer/test/wash. (Wash buffer formulation: 20 mM Tris, 120 mM NaCl, 25 mM MgCl₂)
6. Put filter into scintillation vial containing 5 ml/test of scintillation fluid.
7. Maintain overnight and measure the level of radiation incorporated using a scintillation counter.
8. Generate a standard curve with the concentrations of lipid vs. the level of [³⁵S]GTP S bound.

ORDERING INFORMATION

CATALOG NUMBER
X1572MP

SIZE
50 Tests

STORAGE CUSTOMER

Product should be stored at -20°C. Aliquot to avoid freeze/thaw cycles

STABILITY

Products are stable for one year from purchase when stored properly

SHIP CONDITIONS

Ship on dry ice, freeze upon arrival

FORMULATION

Provided as a sterile solution in 50 mM HEPES containing 2 mM EDTA, 100 mM NaCl and 1 mM MgCl₂ (pH 7.5)

APPLICATIONS

[³⁵S]GTP S Binding Assay

For research use only. Not for use in human diagnostics or therapeutics.

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COMMENTS

Membrane preparation for use in [³⁵S]GTP S binding assays using lysophosphatidic acid as the ligand for the receptor. Prepared from lysed RH7777 cells and contains purified membrane protein with G-protein coupled EDG5 receptors. **NOTE:** The protocol below has been condensed. Please see Ref. 3 for more detailed information.

REFERENCES

1. Goetzl, E.J. and An, S. "Diversity of cellular receptors and functions for the lysophospholipid growth factors lysophosphatidic acid and sphingosine 1-phosphate." FASEB J. 1998, 12, 1589-1598
2. Ancellin, N. and Hla, T. "Differential pharmacological and signal transduction of the sphingosine 1-phosphate receptors EDG-1, EDG-3 and EDG-5." J. Biol. Chem. 1999, 274, 18997-19002
3. Parrill, A.L., et al. "Identification of Edg1 receptor residues that recognize sphingosine 1-phosphate." J. Biol. Chem. 2000, 275, 39379-39384.

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