EDG4 (LPA_2) Functional Receptor Membrane Preparation

Functional Endothelial Cell Differentiation Gene Receptor-4 (LPA_2) Membrane Preparation

BACKGROUND

EDG-4 belongs to a family of G-protein coupled receptors whose ligands are lysophospholipids. The ligand for EDG-4 is lysophospholipid. There are 6 known members of the EDG receptor family and they are implicated in mediating growthrelated 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. EDG4 is expressed in testes, ovarian tumor and leukocyte containing tissues.

ORDERING INFORMATION

CATALOG NUMBER
X1546MP

SIZE
50 Tests

STORAGE CUSTOMER
Product should be stored at -70ºC. 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 $MgCl_2$ (pH 7.5)

APPLICATIONS
[^{35}S]GTPγS Binding Assay

PROTOCOL

1. Add 5-15 µl 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 $MgCl_2$, 10 µM GDP, 2 mM dithiothreitol, 0.1 nM [^{35}S]GTPγS.

2. Add varying quantities of lysophosphatidic acid up to a maximum concentration of 1-2 µM 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 $MgCl_2$)

6. Put filter into scintillation vial containg 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 [^{35}S]GTPγS bound.

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COMMENTS

Membrane preparation for use in [^{35}S]GTPγS 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 EDG4 receptors. **NOTE:** The protocol below has been condensed. Please see Ref. 3 for more detailed information.

REFERENCES

1. Goetzl EJ and An, S. "Diversity of cellular receptors and functions for the lysophospholipid growth factos lysophosphatidic acid and sphingosine 1-phosphate." FASEB J. 1998, 12, 1589-98.

2. An, S., Goetzl, E.J., Lee, H. "Signaling mechanisms and molecular characteristics of G protein-coupled receptors for lysophosphatidic acid and sphingosine 1-phosphate." J. Cell Biochem. Suppl. 1998, 30-31, 147-57.

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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