



## EDG-2 Functional Receptor Membrane Preparation

Functional Endothelial Cell Differentiation Gene Receptor-2 Membrane Preparation

### BACKGROUND

Endothelial cell differentiation gene-2 (EDG-2) receptor is a high affinity receptor for lysophosphatidic acid (LPA). EDG-2, also known as lysophospholipid receptor A1, Vz $\gamma$ -1, mrec1.3 or GPCR26, like all other EDG receptors, couple multiple (3 or more) types of G proteins and transduce decreases in c[AMP]<sub>i</sub> through G<sub>i</sub> and increases in [Ca<sup>2+</sup>]<sub>i</sub> by augmenting phospholipase C through G $\alpha$ q/11 and beta/gamma dimers and by induction of PI3 kinase, p125 FAK, phospholipase D by activating rho through G12/13. Human EDG-2 is present in high levels on oligodendrocytes and certain human malignant T cell lines. EDG-2 receptors (with EDG-4) may play a role in protecting cardiomyocytes from apoptosis induced by hypoxia and andrenergic stimulation.

### ORDERING INFORMATION

**CATALOG NUMBER**  
X1274MP

**SIZE**  
50 Tests

### STORAGE CUSTOMER

Product should be stored at -70 degrees 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 MgCl<sub>2</sub> (pH 7.5)

### APPLICATIONS

GTP- <sup>35</sup>S Binding Assays

### COMMENTS

Membrane preparation for use in GTP- <sup>35</sup>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 EDG-2 receptors. **NOTE:** The protocol below has been condensed. Please see Ref. 4 for more detailed information.

### PROTOCOL

1. Add 5-15  $\mu$ 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 MgCl<sub>2</sub>, 10  $\mu$ M GDP, 2 mM dithiothreitol, 0.1 nM GTP- <sup>35</sup>S).
2. Add varying quantities of lysophosphatidic acid up to a maximum concentration of 1-2  $\mu$ 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 MgCl<sub>2</sub>)
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 GTP- <sup>35</sup>S bound.

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

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## REFERENCES

1. Fukushima, N., et al., "A single receptor encoded by vzg-1/lpA1/edg-2 couples to G proteins and mediates multiple cellular responses to lysophosphatidic acid." Proc. Natl.Acad. Sci. USA 1998, 95, 6151-6156
2. Goetzl, E., et al., "Lysophosphatidic acid and sphingosine 1-phosphate protection of T cells from apoptosis in association with suppression of Bax." J. Immunol. 1999, 162, 2049-2056
3. Zheng, Y., et al. "Lysophosphatidic acid receptor-selection effects on Jurkat T cell migration through a matrigel model basement membrane." J. Immunol. 2001, 166, 2317-2322
4. 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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