CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN LPA_1 LYSOPHOSPHOLIPID RECEPTOR

CATALOG NUMBER: HTS089M **QUANTITY:** 200 units

LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

BACKGROUND:

Lysophosphatidic acid (LPA) is a lysophospholipid produced by activated platelets that inhibits adenylate cyclase and stimulates DNA synthesis, changes in cell morphology, and increases in intracellular calcium in a variety of cultured mammalian cells. A family of three GPCRs, LPA_1, LPA_2 and LPA_3, mediates the biological effects of LPA (Contos *et al.*, 2000). LPA_1 is widely expressed, with particularly notable expression in the ventricular zone of the embryonic cerebral cortex. Mice lacking LPA_1 exhibit craniofacial abnormalities and defective development of the cerebral cortex (Harrison *et al*., 2003; Choi *et al*., 2008). LPA_1 also promotes fibrotic responses to tissue injury (Tager *et al*., 2008)

Millipore's LPA_1 Membrane Preparation is a crude membrane preparation made from the proprietary stable recombinant cell line that is also used for Millipore's LPA_1 Calcium Optimized Cell Line (HTS089C). This LPA_1 Membrane Preparation exhibits both constitutive and agonist-induced activity in GTPγS binding assays; thus it is an ideal HTS tool for screening of compounds for agonist, inverse agonist, and antagonist activities. The membrane preparations exhibits EC50 of 73 nM for LPA in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding Assay

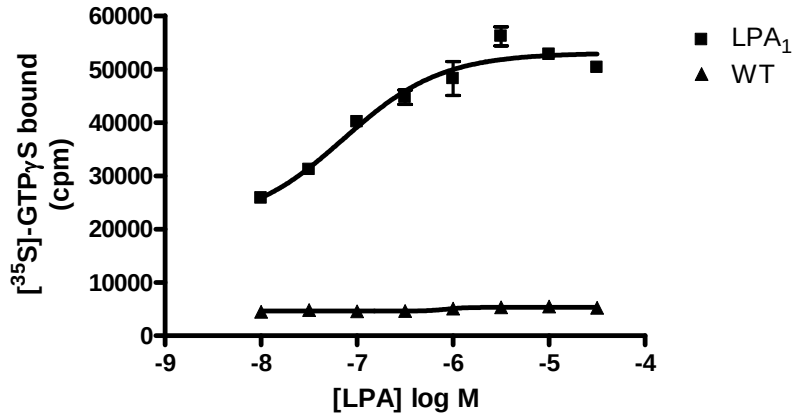

Figure 1. Binding of [^{35}S]-GTPγS to LPA_1 membrane preparation. 5 μg/well LPA_1 Membrane Preparation (catalog # HTS089M) and Wild-type Chem-1 Membrane Preparation (Millipore HTS000MC1) were incubated with 0.3 nM [^{35}S]-GTPγS, 10 μM GDP and increasing amounts of unlabeled LPA. Bound radioactivity was determined by filtration and scintillation counting.

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SPECIFICATIONS: 1 unit = 5 μg
EC50 in GTPγS binding assay by LPA: ~ 73 nM

Species: Human EDG2 encoding LPA_1 (Accession number NM_001401)

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable endogenous LPA receptors.

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GTPγS ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/10 μM GDP in a nonbinding 96-well plate. Unlabeled ligand was added to the final concentration indicated in Figure 1 (final volume 100 μL), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (Millipore MAHF B1H) previously prewetted with water. The plate is washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4, then dried and counted.

PRESENTATION:

One package contains enough membranes for at least 200 assays (units), where one unit is the amount of membrane that will yield greater than 1000 cpm specific LPA-stimulated [^{35}S]-GTPγS binding.

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane protein was adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

Choi JW *et al*. (2008) Biological roles of lysophospholipid receptors revealed by genetic null mice: an update. *Biochim. Biophys. Acta* 1781:531-539.

Contos JJA *et al.* (2000) Lysophosphatidic acid receptors. *Mol. Pharmacol.* 58: 1188-1196.

Harrison SM *et al.* (2003) LPA_1 receptor-deficient mice have phenotypic changes observed in psychiatric disease. *Mol. Cell. Neurosci.* 24: 1170-1179.

Tager AM *et al.* (2008) The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. *Nat. Med.* 14: 45-54.

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