

PRODUCT DATASHEET
ChemiScreen™ S1P₅ Lysophospholipid Receptor Membrane Preparation

CATALOG NUMBER:	HTS193M	QUANTITY:	200 units
LOT NUMBER:	SC20170504	VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND
: Sphingosine 1-phosphate (S1P) is a biologically active lysophospholipid that transmits signals through a family of five G-protein-coupled receptors to regulate cell proliferation, migration, cytoskeletal organization, and differentiation (Spiegel and Milstien, 2003). S1P₅ can couple with Gi/o and G12/13, and it mediates S1P induced adenylate cyclase inhibition and Ca²⁺ mobilization like the other S1P receptors. However, unlike the other S1P receptors, it mediates inhibition of MAPK activation and cell proliferation (Im *et al.*, 2000). S1P₅ is predominantly expressed in the white matter tracts and oligodendrocytes and is particularly abundant in the anterior commissure, corpus colosum, and optic tract (Terai *et al.*, 2003). S1P induces process retraction in pre-oligodendrocytes and supports cell survival in mature oligodendrocytes by activating S1P₅, which indicates a role for S1P₅ in maturation and myelination of oligodendrocytes (Jaillard *et al.*, 2005). Eurofins' S1P₅ membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of S1P₅ interactions with its ligands. The membrane preparations exhibit an EC₅₀ of 5.4 nM for S1P in a GTPγS binding assay.

APPLICATIONS
: GTPγS Binding

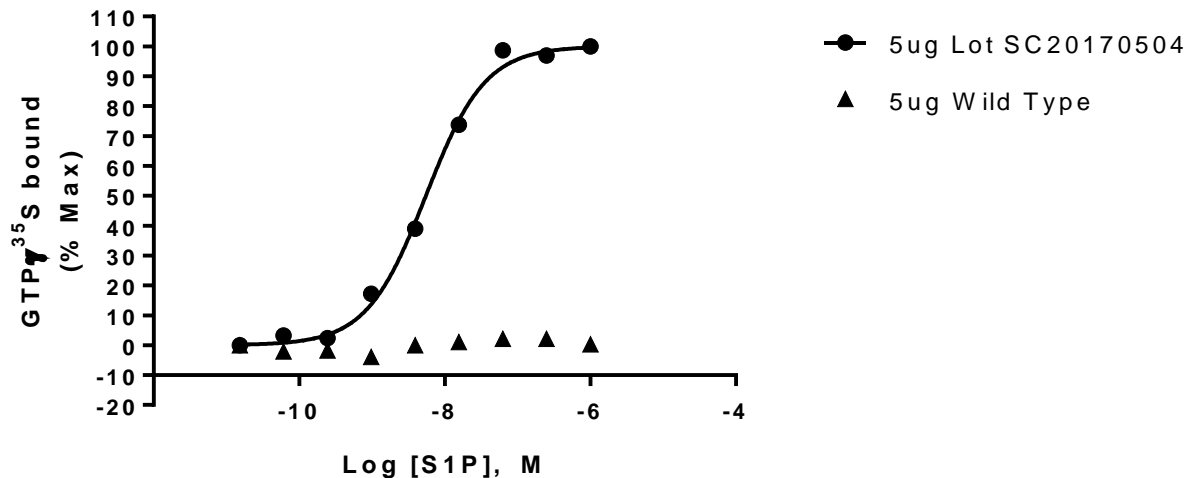


Figure 1. Binding of [³⁵S]-GTPγS to S1P₅ membrane preparation. 5 μg/well S1P₅ Membrane Preparation (catalog # HTS193M) was incubated with 0.3 nM [³⁵S]-GTPγS and increasing amounts of unlabeled S1P. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: 1 unit = 5 µg
EC50 in GTP γ S binding assay by S1P: 5.4 nM

TRANSFECTION: Full-length human EDG8 cDNA encoding S1P₅ (Accession Number: NM_030760)

Species: Human

HOST CELLS: Chem-5, an adherent cell line expressing a promiscuous G-protein.

RECOMMENDED ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [³⁵S]-GTP γ S (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM MgCl₂/0.5 µM GDP in a nonbinding 96-well plate. Unlabeled S1P 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 a GF/B 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.

One vial 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 S1P-stimulated [³⁵S]-GTP γ S binding.

The S1P₅ membrane preparation is expected to be functional in a radioligand binding assay; however, the end user will need to determine the optimal radiolabeled ligand for use with this product.

PRESENTATION: 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 1 mg/ml 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:

1. Im DS *et al.* (2000) Characterization of a novel sphingosine 1-phosphate receptor, Edg-8. *J. Biol. Chem.* 275: 14281-6
2. Jaillard C *et al.* (2005) Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. *J. Neurosci.* 25: 1459-1469.
3. Spiegel S and Milstien S. (2003) Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat. Rev. Mol. Cell Biol.* 4: 397-407.
4. Terai K *et al.* (2003) Edg-8 receptors are preferentially expressed in oligodendrocyte lineage cells of the rat CNS. *Neuroscience* 116: 1053-1062

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

No part of these works may be reproduced in any form without permission in writing.

Eurofins Pharma Bioanalytics Services US Inc. is an independent member of Eurofins Discovery Services