

## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN mGLU<sub>6</sub> METABOTROPIC GLUTAMATE RECEPTOR

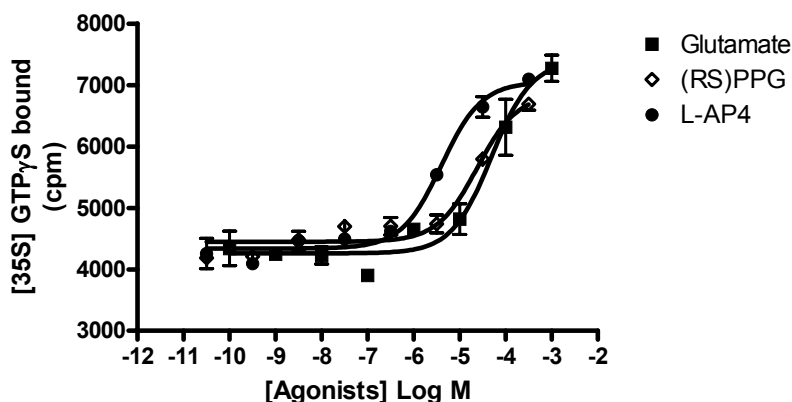
|                        |            |                              |               |
|------------------------|------------|------------------------------|---------------|
| <b>CATALOG NUMBER:</b> | HTS149M    | <b>QUANTITY:</b>             | 200 units     |
| <b>LOT NUMBER:</b>     | R0705E0040 | <b>VOLUME/CONCENTRATION:</b> | 1 mL, 1 mg/mL |

**BACKGROUND:**

Glutamate is a main excitatory neurotransmitter in the central nervous system, and it plays a role in learning, memory and neurotoxicity. The biological actions of glutamate are mediated by ionotropic and metabotropic glutamate receptors, which are ion channels and GPCRs respectively. Metabotropic glutamate receptors (mGluRs) are members of the class 3 G-protein coupled receptor family, which are characterized by a large extracellular domain. They are further classified into group I, II, and III mGluRs on the basis of their sequence identity, pharmacology, and signal transduction mechanism. Group I (mGlu<sub>1</sub> and mGlu<sub>5</sub>) couple to the phospholipase C pathway through G<sub>αq</sub>, whereas group II (mGlu<sub>2</sub> and mGlu<sub>3</sub>) and group III (mGlu<sub>4</sub>, mGlu<sub>6</sub>, mGlu<sub>7</sub>, and mGlu<sub>8</sub>) negatively couple to the adenylyl cyclase pathway through G<sub>αi</sub> (Conn and Pin, 1997). mGlu<sub>6</sub> has a restricted localization to the dendrites of ON bipolar cells that synapse with retinal rods and cones. Targeted deletion of mGlu<sub>6</sub> results in deficits in ON responses but unchanged OFF responses to light (Masu *et al.*, 1995). In addition, mutations in GRM6 in humans are responsible for some forms of autosomal recessive congenital stationary night blindness (Dryja *et al.*, 2005; Zeitz *et al.*, 2005). Chemicon's mGlu<sub>6</sub> 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 mGlu<sub>6</sub> interactions with its ligands. The cell line exhibits a calcium response with EC<sub>50</sub>s of 6.2 μM, 23.4 μM, and 52.7 μM for L-AP4, (RS)PPG, and L-glutamate. The membrane preparations exhibit EC<sub>50</sub>s of 4.0 μM, 24.1 μM, and 49.3 μM for L-AP4, (RS)PPG, and L-glutamate, respectively, in a GTP<sub>γ</sub>S binding assay.

**APPLICATIONS:**

GTP<sub>γ</sub>S Binding and Radioligand Binding Assay.



**Figure 1. Binding of [<sup>35</sup>S]-GTP<sub>γ</sub>S to mGlu<sub>6</sub> membrane preparation.** 5 μg/well mGlu<sub>6</sub> Membrane Preparation (catalog # HTS149M) was incubated with 0.3 nM [<sup>35</sup>S]-GTP<sub>γ</sub>S and increasing amounts of unlabeled L-AP4, (RS) PPG, and glutamate. Bound radioactivity was determined by filtration and scintillation counting.

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SPECIFICATIONS: EC50 in GTP $\gamma$ S binding assay by Glutamate: ~ 49.3  $\mu$ M  
EC50 in GTP $\gamma$ S binding assay by (RS)PPG: ~ 24.1  $\mu$ M  
EC50 in GTP $\gamma$ S binding assay by L-AP4: ~ 4.04  $\mu$ M

Species: human GRM6 cDNA (Accession number NM\_000843)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G $\alpha$ 15.

ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [<sup>35</sup>S]-GTP $\gamma$ S (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM MgCl<sub>2</sub>/0.5  $\mu$ M GDP in a nonbinding 96-well plate. Unlabeled DCG IV, (2R4R) APDC, and glutamate are added to the final concentration indicated in Figure 1 (final volume 100  $\mu$ L), 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, and washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4. The plate is 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 L-AP4, (RS)PPG, or glutamate -stimulated [<sup>35</sup>S]-GTP $\gamma$ S binding.

The mGlu<sub>6</sub> 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:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

## REFERENCES:

Conn PJ and Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* 37: 205-37

Dryja TP *et al.* (2005) Night blindness and abnormal cone electroretinogram ON responses in patients with mutations in the GRM6 gene encoding mGluR6. *Proc. Natl. Acad. Sci. USA* 102(13):4884-9.

Masu M *et al.* (1995) Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. *Cell* 80: 757-65.

Zeit C *et al.* (2005) Mutations in GRM6 cause autosomal recessive congenital stationary night blindness with a distinctive scotopic 15-Hz flicker electroretinogram. *Invest. Ophthalmol. Vis. Sci.* 46: 4328-35.

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