CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN $mGLU_2$ METABOTROPIC GLUTAMATE RECEPTOR

CATALOG NUMBER: HTS146M

QUANTITY: 200 units

LOT NUMBER:

VOLUME/CONCENTRATION: 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 ($mGlu_1$ and $mGlu_5$) couple to the phospholipase C pathway through $G_{\alpha q}$, whereas group II ($mGlu_2$ and $mGlu_3$) and group III ($mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$) negatively couple to the adenylyl cyclase pathway though $G_{\alpha i}$ (Conn and Pin, 1997). Agonists of the Group II metabotropic glutamate receptors, $mGlu_2$ and $mGlu_3$, display efficacy in animal models of anxiety and psychosis. A key role for $mGlu_2$ in mediating these effects is indicated by the observation that selective allosteric potentiator of $mGlu_2$ also retains antipsychotic-like activities in mice (Galici *et al.*, 2005). In addition, $mGlu_{2/3}$ agonists display analgesic activity in animal models (Jones *et al.*, 2005). Chemicon's $mGlu_2$ 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 $mGlu_2$ interactions with its ligands. The cell line exhibits a calcium response with EC50s of 0.51uM, 5.6uM, and 8.3uM for DCG IV, (2R4R) APDC, and glutamate. The membrane preparations exhibit EC50s of of 0.72uM, 5.07uM, and 6.29uM for DCG IV, (2R4R) APDC, and glutamate in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding and Radioligand Binding Assay.

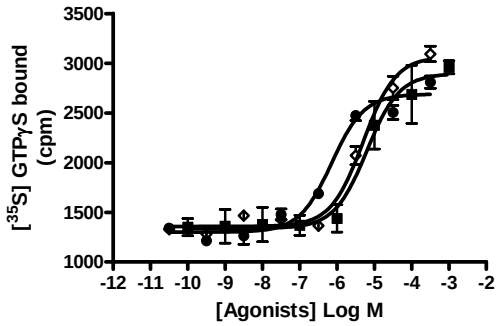

Figure 1. Binding of [^{35}S]-GTPγS to $mGlu_2$ membrane preparation. Representative lot data: 5 µg/well mGlu2 Membrane Preparation (catalog # HTS146M) was incubated with 0.1 nM [^{35}S]-GTPγS and increasing amounts of unlabeled DCG IV, (2R4R) APDC, and glutamate. Bound radioactivity was determined by filtration and scintillation counting.

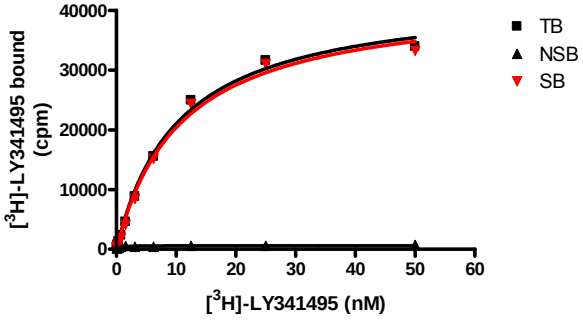

Figure 2. Saturation binding for $mGlu_2$. Representative lot data: 20 µg/well $mGlu_2$ Membrane Preparation was incubated with increasing amount of ^{3}H-labeled LY341495 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled LY341495. Specific binding (SB) was determined by subtracting NSB from TB.

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RECOMBINANT HUMAN $mGLU_2$ METABOTROPIC GLUTAMATE RECEPTOR

SPECIFICATIONS: EC50 in GTPγS binding assay by Glutamate: ~ 6.29 µM
EC50 in GTPγS binding assay by (2R4R) APDC: ~ 5.07 µM
EC50 in GTPγS binding assay by DCG IV: ~ 0.72 µM
Bmax = 8.4 pmol/mg, Kd = 10.8 nM with [^{3}H]-LY341495

Species: human GRM2 cDNA encoding $mGlu_2$ (Accession number NM_000839)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

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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 0.1 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/0.5 µM 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 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, 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.

RADIOLIGAND BINDING ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in assay buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FB 96-well harvest plate (Millipore cat. # MAHF B1H) is prewetted with assay buffer. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with assay buffer. The plate is dried and counted.

Assay buffer: 10 mM potassium phosphate pH 7.6, 100 mM potassium bromide, filtered and stored at 4°C

Radioligand: [^{3}H]-LY341495 (American Radiolabeled Chemicals ART1439)

PRESENTATION:

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 DCG IV, (2R4R) APDC, or glutamate -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:

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

Galici R *et al.* (2005) A selective allosteric potentiator of metabotropic glutamate (mGlu) 2 receptors has effects similar to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of antipsychotic activity. *J. Pharmacol. Exp. Ther.* 315(3):1181-7

Jones CK *et al.* (2005) Analgesic effects of the selective group II (mGlu2/3) metabotropic glutamate receptor agonists LY379268 and LY389795 in persistent and inflammatory pain models after acute and repeated dosing. *Neuropharmacology* 49 Suppl 1:206-18.

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