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PRODUCT DATASHEET

ChemiScreen™ Recombinant Human $mGlu_2$ Metabotropic Glutamate Receptor Membrane Preparation

CATALOG NUMBER:	HTS146M	**QUANTITY:**	200 units
LOT NUMBER:	21K1202	**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). $mGlu_2$ 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.

APPLICATIONS:

Radioligand binding assay

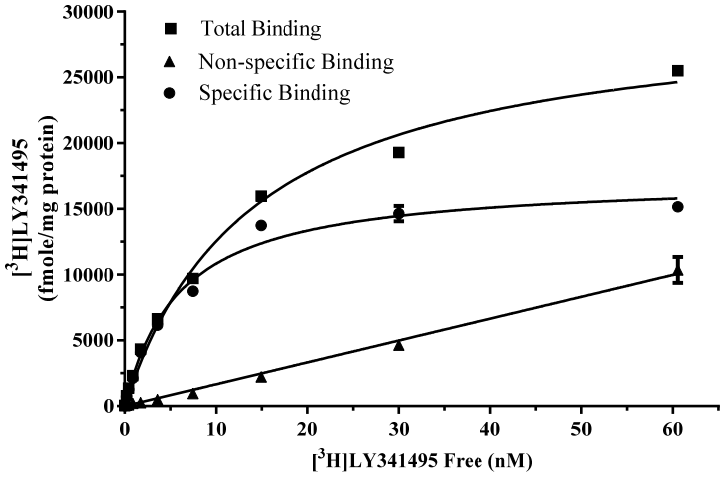

Figure 1. Saturation binding for $mGlu_2$ membrane preparation. 2 µg/well mGlu2 Membrane Preparation was incubated with increasing amount of 3H-labeled LY341495 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of unlabeled LY-354740 at 10µM. Specific binding (SB) was determined by subtracting NSB from TB.

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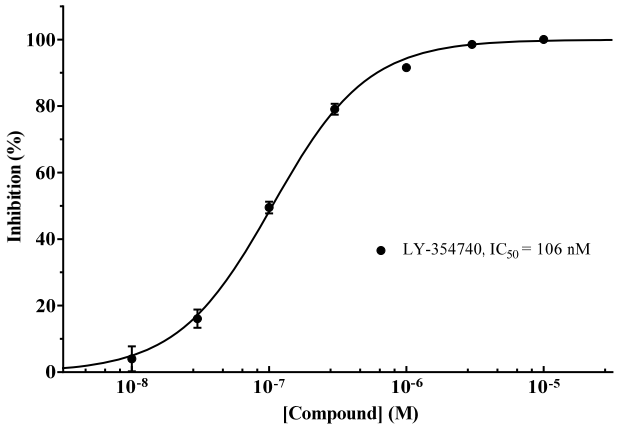

Figure 2. Competition binding for mGlu$_2$ membrane preparation. 2 µg/well mGlu2 Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 2 nM 3H-labeled LY341495 and increasing concentrations of unlabeled LY-354740. More than 10- fold signal:background was obtained with LY-354740.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{3}H] LY341495 binding: 17.4 pmol/mg protein
K_d for [^{3}H] LY341495 binding: ~6.0 nM
Signal:background: >10-fold

TRANSFECTION: Human GRM2 cDNA encoding mGlu$_2$ (Accession number NM_000839)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15 and with no endogenous expression of mGlu2.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1h. Prior to filtration, an GF/B 96-well harvest plate is coated with 10 mM KH_2PO_4, 100 mM KBr, pH 7.6. Binding reaction is transferred to the filter plate, and washed 4 times (~1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 10 mM KH_2PO_4, 100 mM KBr, pH 7.6

Radioligand: [3H]-LY341495 (ARC, ART1439)

Wash Buffer: 10 mM KH_2PO_4, 100 mM KBr, pH 7.6

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 10-fold signal:background with ^{3}H-labeled LY341495.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml 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. Conn PJ and Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* 37: 205-37
2. 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
3. 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.