

CHEMISCREEN™ MEMBRANE PREPARATION HUMAN RECOMBINANT GAL₂ RECEPTOR

CATALOG NUMBER: HTS186M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 2 mL, 1 mg/mL

BACKGROUND: Galanin is a 29-30 amino acid peptide originally purified from intestine, but later found to be abundant in the CNS. It is widely distributed in tissues such as the brain, spinal cord and gut, and can regulate numerous processes including feeding, nociception, nerve regeneration, memory, neuroendocrine release, and gut secretion and contractility. Galanin elicits its physiological effects through the stimulation of at least three G protein-coupled receptors (Branchek *et al.* 2000). GAL₂ receptor couples predominantly to the activation of phospholipase C. It plays an important role in modulating neurite outgrowth and has been demonstrated to be the principal receptor subtype that mediates the protective effects of galanin in the hippocampus (Elliott-Hunt *et al.* 2007). Millipore's GAL₂ receptor 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 antagonists of GAL₂ receptor interactions. The membrane preparations exhibit a K_d of 1.4 nM for [¹²⁵I]-Galanin. With 10 μg/well GAL₂ receptor Membrane Prep and 0.5 nM [¹²⁵I]-Galanin, a greater than 20-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay, and GTPγS binding.

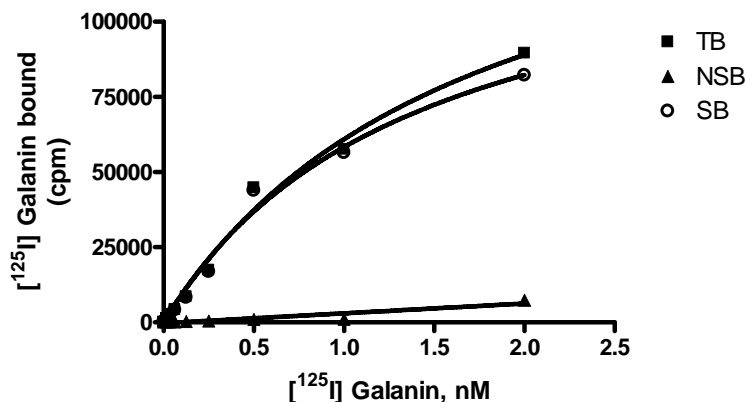


Figure 1. Saturation binding for GAL₂ receptor. 10 μg/well GAL₂ receptor Membrane Preparation was incubated with increasing amount of [¹²⁵I]-Galanin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Galanin (2-29). Specific binding (SB) was determined by subtracting NSB from TB.

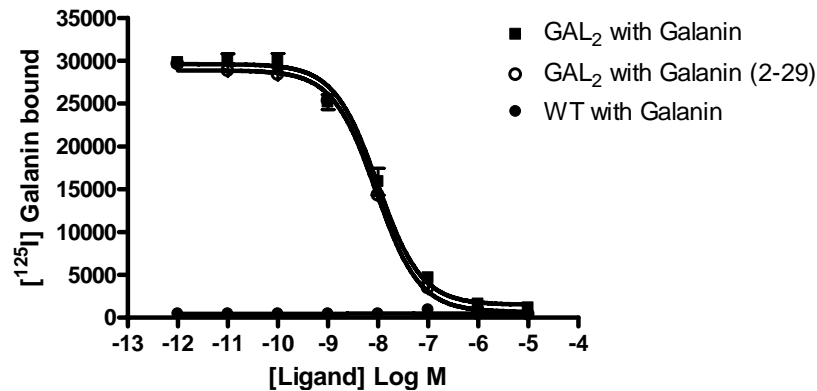


Figure 2. Competition binding for GAL₂ receptor. GAL₂ receptor Membrane Preparation (10µg/well) or Wild-Type Chem-1 membrane preparation was incubated with 0.5 nM [¹²⁵I]-Galanin and increasing concentrations of unlabeled cold ligand, and more than 20-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with GAL₂ receptor membrane prep and cold galanin (2-29).

	10 µg/well
Signal:background	42.4
Specific binding (cpm)	28192

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
 Bmax: 8.2 pmol/mg
 K_d: ~1.4 nM

Species: Full-length human GALR2 cDNA encoding GAL₂ (Accession Number: NM_003857)

HOST CELLS: Chem-1, a adherent mammalian cell line without any endogenous GAL₂ receptor expression.

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 1-2 h. Prior to filtration, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 50 mM Hepes, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, filtered and stored at 4°C

Radioligand: [¹²⁵I] galanin (Perkin Elmer # NEX333)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, filtered and stored at 4°C.



One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 20-fold signal:background with ¹²⁵I-labeled galanin at 0.5 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membranes protein were adjusted to the indicated concentration 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:

Branchek TA, Smith KE, Gerald C, Walker MW (2000) Galanin receptor subtypes. *Trends Pharmacol. Sci.* 21: 109-17.

Elliott-Hunt CR, Pope RJ, Vanderplank P, Wynick D (2007) Activation of the galanin receptor 2 (GalR2) protects the hippocampus from neuronal damage. *J. Neurochem.* 100: 780-9.

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