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PRODUCT DATASHEET

ChemiScreen™ Human Recombinant Gal2 Receptor Membrane Preparation

CATALOG NUMBER:	HTS186M	**QUANTITY:**	200 units
LOT NUMBER:	SC886297	**VOLUME/CONCENTRATION:**	1 mL, 2 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). Eurofin'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 Kd of 0.813 nM for [^{125}I]-Galanin. With 10 μg/well GAL2 receptor Membrane Prep and 0.5 nM [^{125}I]-Galanin, a greater than 5-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand Binding Assay

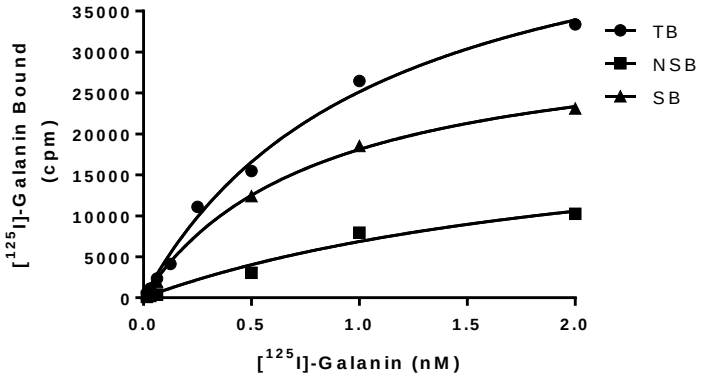

Figure 1. Saturation Binding for GAL_2 Receptor. 10 μg/well of GAL_2 receptor Membrane Preparation were incubated with increasing amounts of [^{125}I]-Galanin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of a 200-fold excess of unlabeled Galanin (2-29). Specific binding (SB) was determined by subtracting NSB from TB.

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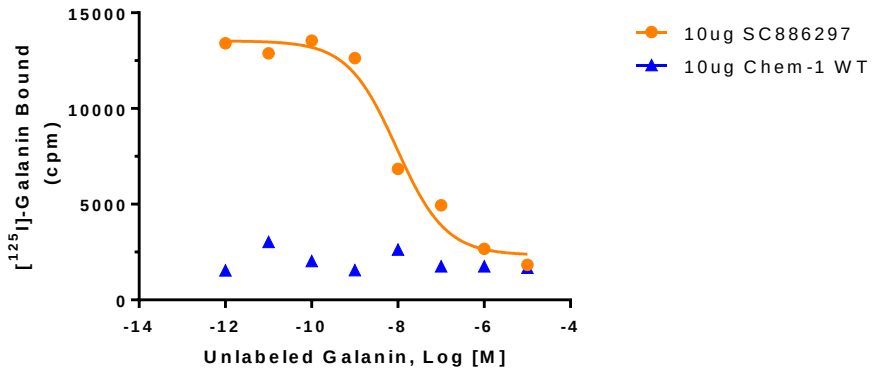

Figure 2. Competition Binding for GAL2 Receptor. GAL2 Receptor Membrane Preparation (10 μg/well) or Wild-Type Chem-1 Membrane Preparation was incubated with 0.5 nM [^{125}I]-Galanin and increasing concentrations of unlabeled cold ligand. A greater than 5-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 10 μg membrane preparation
Bmax: 0.95 pmol/mg
K_d: 0.813 nM
Signal:background: >5-fold

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

Species: Human

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

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 2 h. Prior to filtration, an FC 96-well harvest plate (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4. The 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 $MgCl_2$, 1 mM $CaCl_2$, filtered and stored at 4°C

Radioligand: [^{125}I]-Galanin (Perkin Elmer # NEX333)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM 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 5-fold signal:background with [^{125}I]-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: Membrane proteins were adjusted to the indicated concentration in 1 mL packaging buffer, rapidly frozen, and stored at -80^{o}C.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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:

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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