

PRODUCT DATASHEET
ChemiScreen™ GAL₁ Galanin Receptor Membrane Preparation

CATALOG NUMBER:	HTS094M	QUANTITY:	200 units
LOT NUMBER:	SC20170125	VOLUME/CONCENTRATION:	1 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. A family of 3 GPCRs, GAL1, GAL2 and GAL3, bind to galanin and mediate its biological effects. Galanin and its receptors have been implicated in pain, cognition, seizure activity, depression and drug addiction (Hökfelt, 2005). In particular, GAL1 mediates the anticonvulsant activity of galanin; mice lacking GAL1 are affected by spontaneous seizures, and have increased susceptibility to seizures induced by lithium-pilocarpine treatment and electrical perforant path stimulation (McColl et al., 2006; Mazarati et al., 2004). Chemicon's GAL1 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 GAL1 interactions with galanin.

APPLICATIONS: Radioligand binding assay

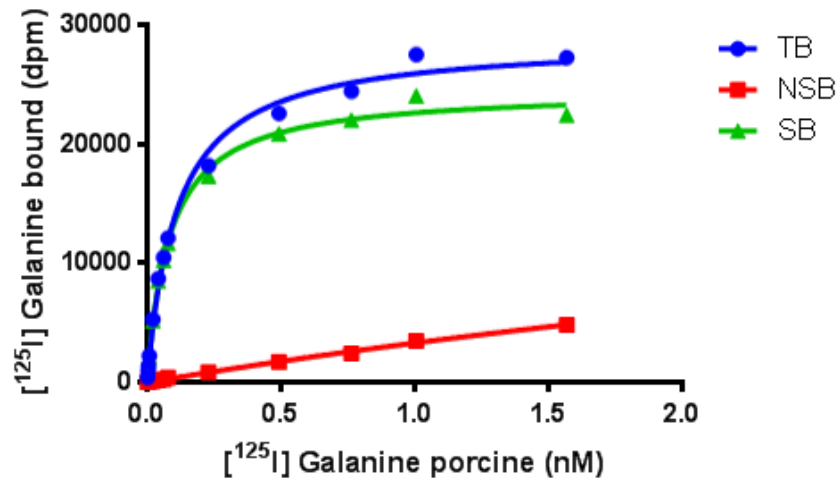


Figure 1. Saturation binding for GAL1. 0.25µg/well GAL1 Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled Galanine porcine in the absence (total binding, TB) or presence (no specific binding, NSB) of unlabeled Galanine porcine at 1 µM. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

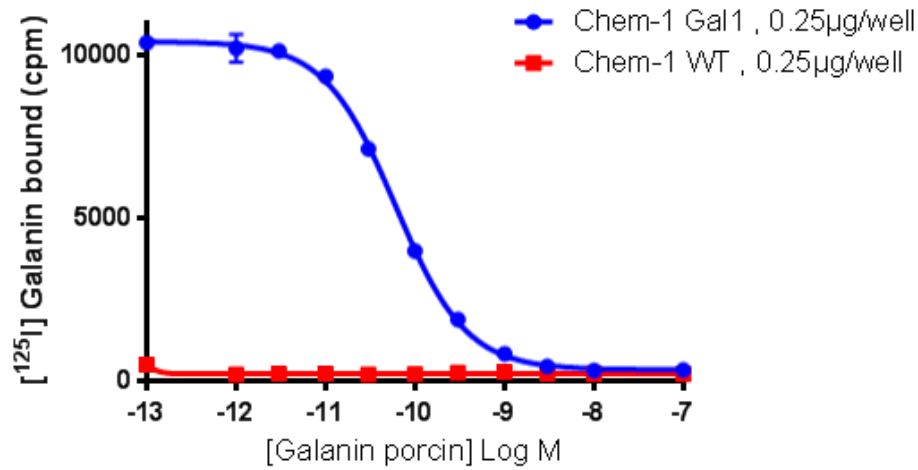


Figure 2. Competition binding for Gal1. 0.25 µg/well Gal1 and 0.25 µg/well wild type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.04 nM ¹²⁵I-labeled Galanin porcine and increasing concentrations of unlabeled Galanin porcine. More than 10-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg

B_{max} for [¹²⁵I]-Galanin porcine binding: 28 980 fmol/mg protein

K_d for [¹²⁵I]-Galanin porcine binding: ~0.085 nM

Signal:background: >10-fold

TRANSFECTION: Human GAL1 (Accession number NM_001480)

Species : Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous GAL1 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 1h. Prior to filtration, a GF/B 96-well harvest plate is coated with 50 mM Tris-HCl, pH 7.4 + 0.3% polyethyleneimine. 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: 50 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 0.5% BSA stored at 4°C.

Radioligand: [¹²⁵I]-Galanin porcine. (Chelatec # RL20)

Wash Buffer: 50 mM Tris-HCl, pH 7.4.

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 ¹²⁵I labeled Galanin porcine.

- 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. Hökfelt T (2005) Galanin and its receptors: Introduction to the Third International Symposium, San Diego, California, USA, 21-22 October 2004. *Neuropeptides* 39: 125-142.
 2. Mazarati A et al. (2004) Patterns of seizures, hippocampal injury and neurogenesis in three models of status epilepticus in galanin receptor type 1 (GalR1) knockout mice. *Neuroscience* 128: 431-441.
 3. McColl CD et al. (2006) Galanin receptor-1 knockout mice exhibit spontaneous epilepsy, abnormal EEGs and altered inhibition in the hippocampus. *Neuropharmacology* 50: 209-18

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