

PRODUCT DATASHEET
ChemiScreen™ 5-HT_{4B} Serotonin Membrane Preparation

CATALOG NUMBER:	HTS110M	QUANTITY:	200 units
LOT NUMBER:	22L2906	VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND: 5-Hydroxytryptamine (5-HT, also commonly known as serotonin) is synthesized in enterochromaffin cells in the intestine and in serotonergic nerve terminals. In the periphery, 5-HT mediates gastrointestinal motility, platelet aggregation, and contraction of blood vessels. Many functions of the central nervous system are influenced by 5-HT, including sleep, motor activity, sensory perception, arousal and appetite. A family of 12 GPCRs and one ion channel mediate the biological effects of 5-HT (Hoyer *et al.*, 1994). 5-HT₄ comprises at least 8 isoforms varying at the C-terminus, which are generated by alternative splicing. The expression and distribution of these splice variants differs among organs and tissues with many of them present in several tissues such as atrium, brain, and GI tract (Bockaert *et al.* 2004). To date, all isoforms have been shown to activate adenylyl cyclase *in vitro*, and no difference in signal transduction between C-terminal 5-HT₄ receptor variants has been demonstrated. 5-HT₄ receptors are of potential interest for the treatment of patients with GI motility disorders and Alzheimer's disease (De Maeyer *et al.* 2008; Lezoualc'h 2007). 5-HT_{4B} 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 agonists and antagonists of 5-HT_{4B}.

APPLICATIONS: Radioligand binding assay

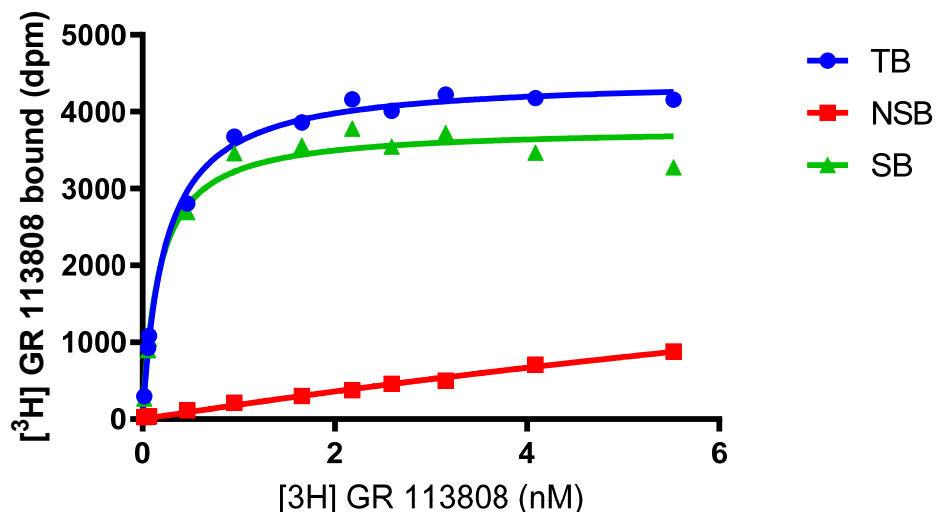


Figure 1. Saturation binding for 5-HT_{4B}. 5 µg/well of 5-HT_{4B} Membrane Preparation were incubated with increasing amounts of [³H]-GR113808 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess of unlabeled GR113808. Specific binding (SB) was determined by subtracting NSB from TB. The sample data are from a representative lot.

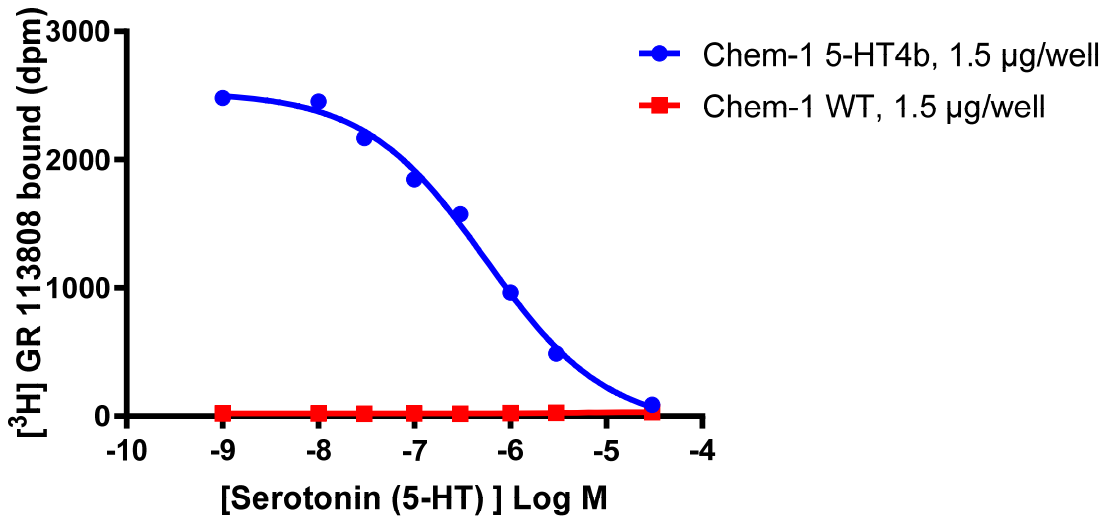


Figure 2. Competition binding for 5-HT_{4B}. 5-HT_{4B} Membrane Preparation 1.5 µg/well and 1.5 µg/well Wild-Type Chem-1 membrane preparation (WT, catalog # HTS000MC1) were incubated with 1 nM [³H]-GR113808 and increasing concentrations of unlabeled serotonin, and more than a 8-fold signal:background ratio was obtained. The sample data are from a representative lot.

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
 B_{max} for [³H]-GR113808 Binding: 14.0 pmol/mg protein
 K_d for [³H]-GR113808 Binding: 0.17 nM
 Signal:background: >6

TRANSFECTION: Human HTR4, transcript b, encoding 5-HT_{4B} (Accession number NM_000870)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable endogenous 5-HT_{4B} expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in assay buffer in a non-binding 96-well plate, and incubated at room temperature for 2 h. Prior to filtration, an FC 96-well harvest plate is coated with 0.33% polyethyleneimine for 30 min, then washed with Assay Buffer. The binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Assay Buffer. The wells are then dried and counted.

Assay Buffer: 25 mM Tris, pH 7.4, filtered and stored at 4°C

Radioligand: [³H]-GR113808 (PerkinElmer#: NET1152)

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 6-fold signal:background with [³H]-GR113808 at 1 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°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. Hoyer D *et al.* (1994). International Union of Pharmacology classification of receptors for 5-HT hydroxytryptamin (Serotonin). *Pharmacol Rev.* 46(2):157-203.
2. Bockaert J *et al.* (2004). 5-HT₄ receptors. *Curr Drug Targets CNS Neurol Disord.* 3:39-51.
3. De Maeyer JH *et al.* (2008). 5-HT₄ receptor agonists: similar but not the same. *Neurogastroenterol Motil* 20(2):99-112.
4. Lezoualc'h F (2007). 5-HT₄ receptor and Alzheimer's disease: the amyloid connection. *Exp Neurol* 205:325-329.

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