

CHEMISCREEN™ MEMBRANE PREPARATION HUMAN RECOMBINANT 5-HT_{1B} SEROTONIN RECEPTOR

CATALOG NUMBER:	HTS108M	QUANTITY:	200 units
LOT NUMBER:	R0711E0002	VOLUME/CONCENTRATION PER VIAL:	2 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). The 5-HT_{1B} receptor (also known as 5-HT_{1Dβ}) is expressed presynaptically on serotonergic neurons and postsynaptically on non-serotonergic neurons, and it regulates release of 5-HT and acetylcholine. Several brain regions, including globus pallidus, substantia nigra and dorsal subiculum, highly express HT_{1B}. 5-HT_{1B} has been implicated in the physiology of aggression, depression, migraine, anxiety and reward for drugs of abuse (Sari, 2004). 5-HT_{1B} interacts with p11, which increases cell surface expression and function of 5-HT_{1B} (Svenningsson *et al.*, 2006). Millipore's 5-HT_{1B} 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_{1B}. The membrane preparations exhibit a K_d of 10.5 nM for [³H]-GR125743. With 7 nM [³H]-GR125743, 10 μg/well 5-HT_{1B} Membrane Prep typically yields greater than 8-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay and GTPγS binding.

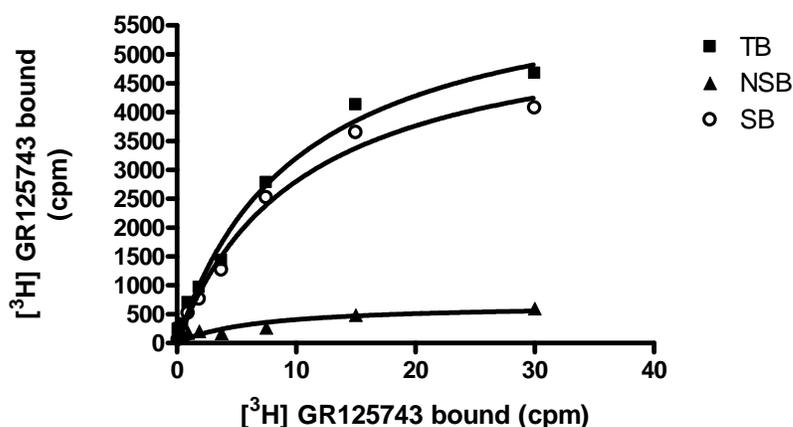


Figure 1. Saturation binding for 5-HT_{1B}. 10 μg/well 5-HT_{1B} Membrane Preparation was incubated with increasing amount of ³H-labeled GR125743 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled GR55562. Specific binding (SB) was determined by subtracting NSB from TB.

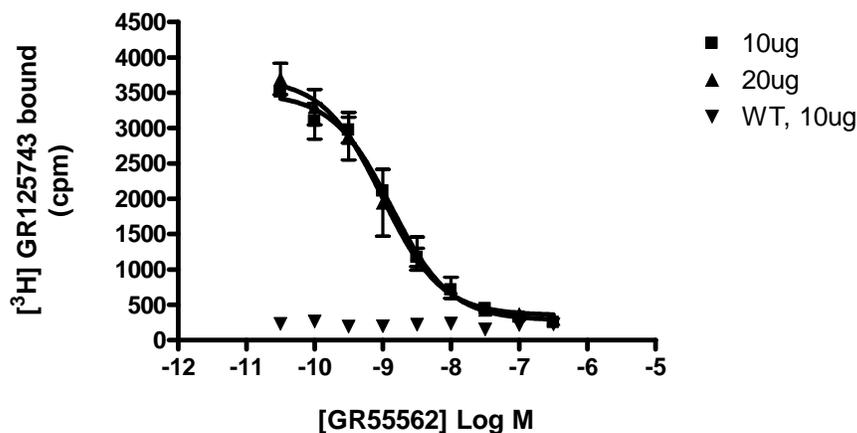


Figure 2. Competition binding for 5-HT_{1B}. 10 and 20µg/well 5-HT_{1B} Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 7 nM ³H-labeled GR125743 and increasing concentrations of unlabeled GR55562. More than 8-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of 5-HT_{1B} Receptor membrane prep.

	10 µg/well	20 µg/well
Signal:background	12.4	10.5
Specific binding (cpm)	3210	3352

SPECIFICATIONS: 1 unit = 10 µg
 B_{max} for [³H]-GR125743 binding: 3.7 pmol/mg protein
 K_d for [³H]-GR125743 binding: ~10.5 nM

TRANSFECTION: Full-length human HTR1B cDNA encoding the 5-HT_{1B} Serotonin Receptor (Accession Number: NM_000863)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous 5-HT_{1B} 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 Tris, 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 Tris, pH 7.4, 10 mM MgCl₂, 1 mM EDTA, filtered and stored at 4°C

Radioligand: [³H]-GR125743. (Amersham #:TRK-1046)

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

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 8-fold signal:background with ³H labeled GR125743 at 7 nM

- PRESENTATION:** Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes protein were adjusted to 1 mg/ml in 1 ml 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:**
- Hoyer D *et al.* (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.* 46: 157-203.
- Sari Y (2004) Serotonin_{1B} receptors: from protein to physiological function and behavior. *Neurosci. Biobehav. Rev.* 28: 565-82.
- Svenningsson P *et al.* (2006) Alterations in 5-HT_{1B} receptor function by p11 in depression-like states. *Science* 311: 77-80.

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