

PRODUCT DATASHEET
ChemiScreen™ 5-HT_{1B} Serotonin Membrane Preparation

CATALOG NUMBER:	HTS108M	QUANTITY:	200 units
LOT NUMBER:	22H2409	VOLUME/CONCENTRATION:	1 mL, 2.0 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). 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}.

APPLICATIONS: Radioligand binding assay

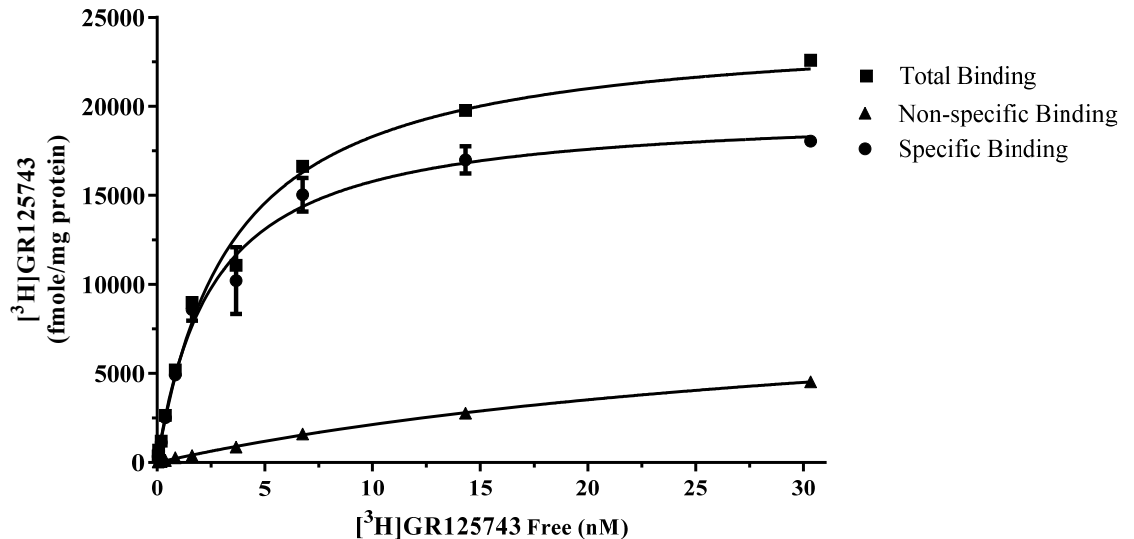


Figure 1. Saturation binding for 5-HT_{1B}. 10 μg/well 5-HT_{1B} Membrane Preparation was incubated with increasing amount of ³H-labeled GR125743 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 10 μM unlabeled GR55562. Specific binding (SB) was determined by subtracting NSB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

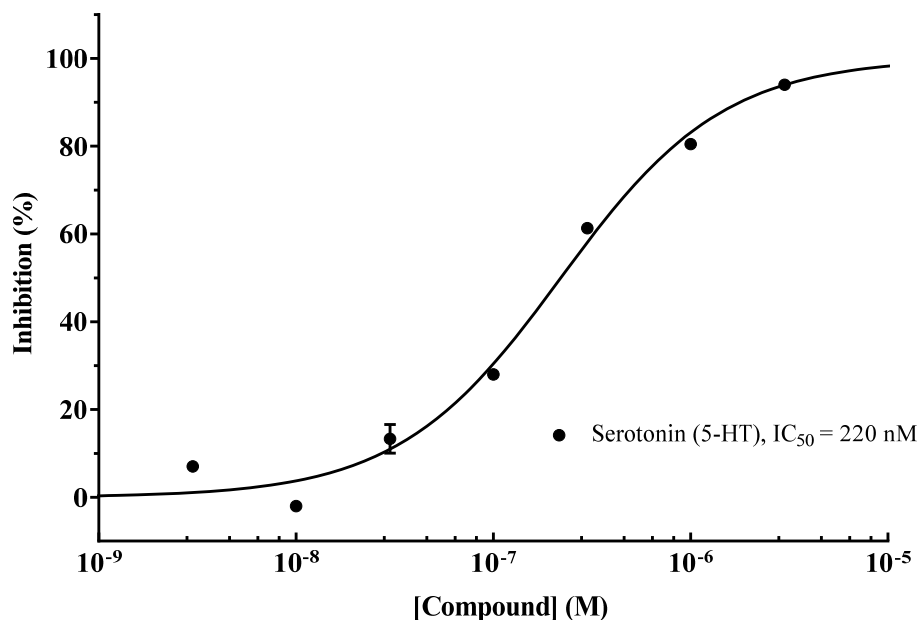


Figure 2. Competition binding for 5-HT_{1B}. 10µg/well 5-HT_{1B} Membrane Preparation was incubated in a 96-well plate with 1 nM ³H-labeled GR125743 and increasing concentrations of unlabeled GR55562. More than 3-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 10 µg
 B_{max} for [³H]-GR125743 binding: 19.8 pmol/mg protein
 K_d for [³H]-GR125743 binding: 2.6 nM
 Signal:Background: >3-fold

Species: Human 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 (EMD 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. (Perkin Elmer #: NET1172)

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 with no preservatives.
Packaging method: Membranes protein was adjusted to the indicated concentration in 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-hydroxytryptamine (Serotonin). *Pharmacol. Rev.* 46: 157-203.
2. Sari Y (2004) Serotonin_{1B} receptors: from protein to physiological function and behavior. *Neurosci. Biobehav. Rev.* 28: 565-82.
3. 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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