

CATALOG NUMBER: HTS141M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 2 mL, 1 mg/mL

BACKGROUND:

The neurotransmitter serotonin/5-hydroxytryptamine (5-HT) regulates a wide variety of neurological functions. A family of 13 receptors (12 GPCRs and one ion channel) mediate the effects of serotonin (Hoyer *et al.*, 1994). The serotonin receptors 5-HT_{5A} and 5-HT_{5B} are G_{i/o} coupled receptors mainly expressed in the CNS but not in peripheral organs. 5-HT_{5A} is found in rodents and humans and is broadly expressed in the CNS whereas 5-HT_{5B} exists in rodents, but not humans, and is expressed in a more restricted fashion (Grailhe *et al.*, 2001). The hallucinogenic drug LSD functions as a partial agonist at 5-HT_{5A}, and mice lacking 5-HT_{5A} display reduced LSD-induced locomotion (Nelson 2004). HT_{5A} receptors may also be involved in a neuronally-driven mechanism for regulating astrocyte physiology, with relevance to gliosis (Glennon *et al.*, 2000).

Millipore's 5-HT_{5A} 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 5-HT_{5A} interactions and its ligands. The membrane preparations exhibit a K_d of 67.7 nM for [³H]-5-CT (Carboxamidotryptamine). With 30 nM [³H]- 5-CT, 10 µg/well 5-HT_{5A} Membrane Prep yields greater than 3-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

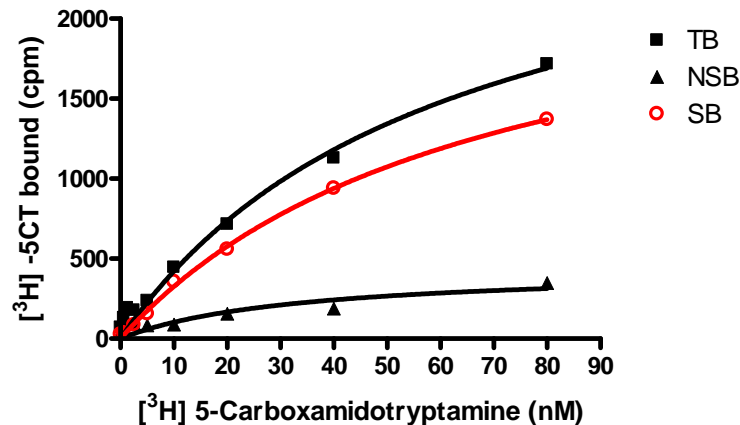


Figure 1. Saturation binding for 5-HT_{5A}. 10.0 µg/well 5-HT_{5A} Membrane Preparation was incubated with increasing amount of [³H]-5-CT in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled 5-CT. Specific binding (SB) was determined by subtracting NSB from TB.

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

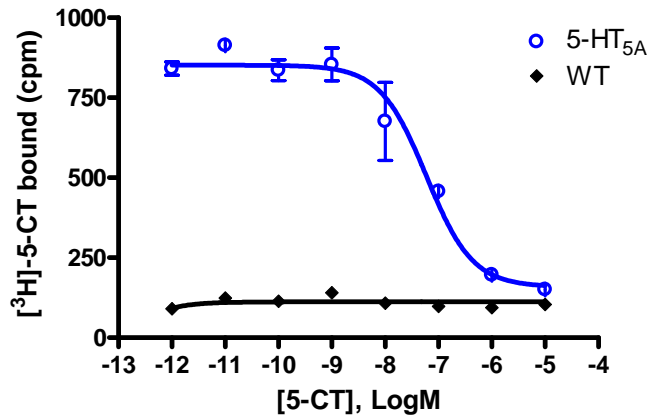


Figure 2. Competition binding for 5-HT_{5A}. 5-HT_{5A} Membrane Preparation and Wild-Type Chem-1 membrane preparation (Millipore cat. # HTS000MC1), each 10 µg/well, were incubated with 30.0 nM [³H]-5-CT and increasing concentrations of unlabeled 5-CT, and more than 3-fold signal: background was obtained.

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

	10 ug/well
Signal: background	5.4
Specific binding (cpm)	693

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
 B_{max}: 28.7 pmol/mg
 K_d: 67.7 nM

Species: Human 5-HT_{5A} (Accession number NM_024012)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous 5-HT_{5A} 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 HEPES, 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 HEPES, pH 7.4, 5 mM MgCl₂, 4 mM CaCl₂, 0.5 mM Ascorbic Acid filtered and stored at 4°C

Radioligand: [³H]-5-Carboxamidotryptamine (Perkin Elmer #:NET1071)

Wash Buffer: 50 mM HEPES, pH 7.4, 5 mM MgCl₂, 4 mM CaCl₂, 0.5 mM Ascorbic Acid, filtered and stored at 4°C.



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One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 3-fold signal: background with ³H-labeled 5-carboxamidotryptamine at 30.0 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membrane 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:

Glennon R *et al.* (2000) Serotonin Receptor Subtypes and Ligands. *Psychopharmacology: The Fourth Generation of Progress*.

Grailhe R *et al.* (2001) Human 5-HT₅ receptors: the 5-HT_{5A} receptor is functional but the 5-HT_{5B} receptor was lost during mammalian evolution. *Eur. J. Pharmacol.* 418: 157-167.

Hoyer D *et al.* (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.* 46: 157 - 203.

Nelson DL (2004) 5-HT₅ receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3: 53-58.

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