

**CHEMISCREEN™ MEMBRANE PREPARATION
HUMAN RECOMBINANT 5-HT₇ SEROTONIN RECEPTOR**

CATALOG NUMBER:	HTS215M	QUANTITY:	200 units
LOT NUMBER:	RI08040036	VOLUME/CONCENTRATION PER VIAL:	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. The serotonin receptor 5-HT₇ is a Gs coupled receptor expressed in the brain (hypothalamus, hippocampus and frontal cortex) and in smooth muscle and GI tract. Systemic delivery of serotonin results in hypothermia, and pharmacological and genetic studies indicate that 5-HT₇ mediates this effect (Hedlund *et al.*, 2003). In addition, 5-HT₇ appears to modulate circadian rhythms and smooth muscle relaxation, and is implicated as a target for sleep, particularly as related to depression, and migraine (Terron and Falcon-Neri, 1999; Mullins *et al.*, 1999). Millipore's 5-HT₇ 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-HT₇. The membrane preparations exhibit a K_d of 37.7 nM for [³H]-5-Carboxamidotryptamine (5-CT). With 30 nM [³H]-5-CT, 10 µg/well 5-HT₇ Membrane Prep typically yields greater than 4-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

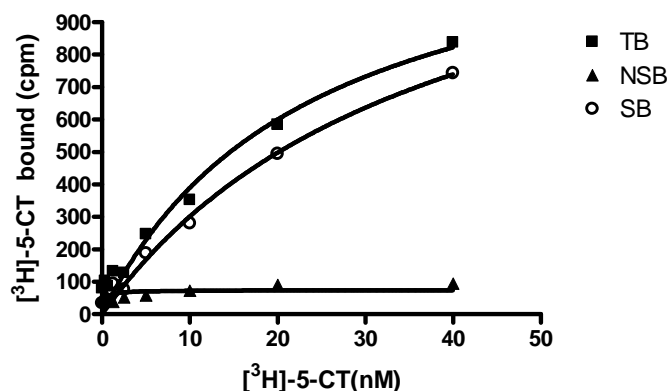


Figure 1. Saturation binding for 5-HT₇. 10 µg/well 5-HT₇ Membrane Preparation was incubated with increasing amount of ³H-labeled 5-CT in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled 5-CT. Specific binding (SB) was determined by subtracting NSB from TB.

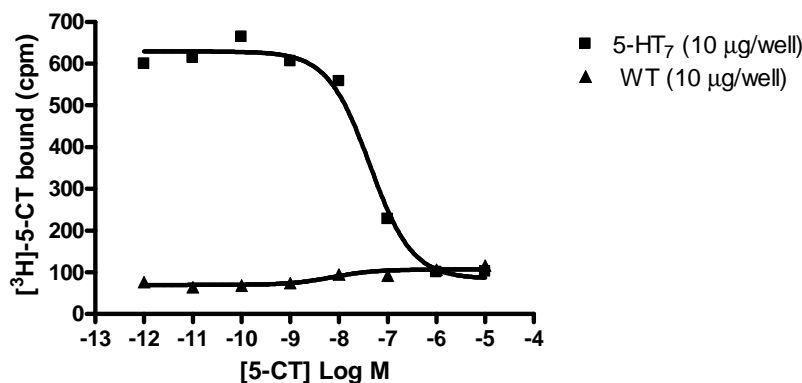


Figure 2. Competition binding for 5-HT₇. 5-HT₇ Membrane Preparation (HTS215M) and wild-type Chem-1 Membrane Preparation (Millipore catalog # HTS000MC1), each at 10 µg/well, were incubated in a 96-well plate with 30 nM ³H-labeled 5-carboxamidotryptamine (5-CT) and increasing concentrations of unlabeled (5-CT). More than 4-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with 10 µg of 5-HT₇ Receptor membrane prep.

	10 µg/well
Signal:background	7.5
Specific binding (cpm)	545

SPECIFICATIONS: 1 unit = 10 µg
 B_{max} for [³H]-5-CT binding: 20.1 pmol/mg protein
 K_d for [³H]-5-CT binding: ~37.7 nM

TRANSFECTION: Full-length human HTR7 cDNA encoding the 5-HT₇ Serotonin Receptor (Accession Number: NM_000872)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous 5-HT₇ 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 the binding buffer. 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, 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: same as the binding buffer.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 4-fold signal:background with ³H-labeled 5-CT at 30 nM

PRESENTATION:

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

Packaging method: Membranes protein were adjusted to the indicated concentration in 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:

Hedlund PB *et al.* (2003) No hypothermic response to serotonin in 5-HT₇ receptor knockout mice. *Proc. Natl. Acad. Sci. USA* 100: 1375-80.

Terron JA and Falcon-Neri A (1999) Pharmacological evidence for the 5-HT₇ receptor mediating smooth muscle relaxation in canine cerebral arteries. *Br. J. Pharmacol.* 127: 609-16

Mullions UL *et al.* (1999) Effects of antidepressants on 5-HT₇ receptor regulation in the rat hypothalamus. *Neuropsychopharmacology* 21: 352-67

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