



CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN 5-HT₆ SEROTONIN RECEPTOR

CATALOG NUMBER: HTS111M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 1 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-HT₆ is a G_s coupled receptor expressed solely in the CNS, primarily in the limbic and cortical regions. 5-HT₆ appears to play a role in memory and learning, obesity, psychosis, anxiety and epilepsy (Woolley *et al.*, 2004; Fisas *et al.*, 2006). In particular, a 5-HT₆-selective agonist caused significant weight loss in a rat model of diet-induced obesity. Millipore's 5-HT₆ 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-HT₆. The membrane preparations exhibit a K_d of 0.44 nM for [¹²⁵I]-SB258585. With 0.25nM [¹²⁵I]-SB258585, 5µg/well 5-HT₆ Membrane Prep typically yields greater than 6-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

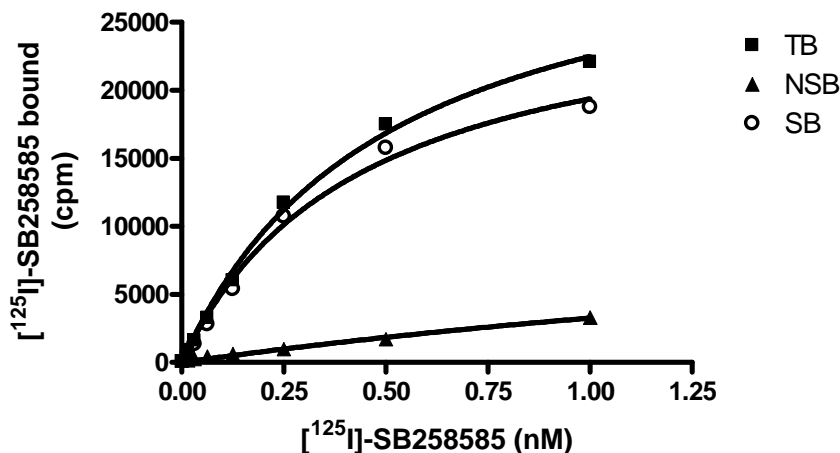


Figure 1. Saturation binding for 5-HT₆. 5 µg/well 5-HT₆ Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled SB-258585 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled 5-HT. Specific binding (SB) was determined by subtracting NSB from TB.

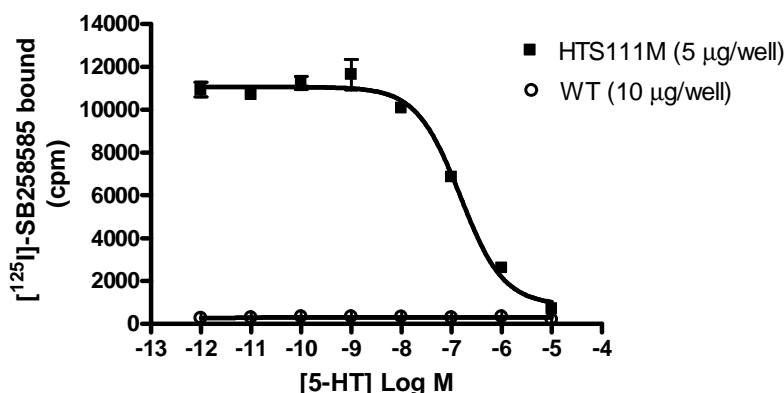


Figure 2. Competition binding for 5-HT₆. 5-HT₆ Membrane Preparation (5 µg/well) and wild-type Chem-1 Membrane Preparation (10 µg/well; Millipore catalog # HTS000MC1) were incubated in a 96-well plate with 0.25 nM ¹²⁵I-labeled SB258585 and increasing concentrations of unlabeled 5-HT. More than 6-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of 5-HT₆ Receptor membrane prep.

	10 µg/well	5 µg/well
Signal:background	12.3	12.8
Specific binding (cpm)	12675	10192

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [¹²⁵I]-SB258585 binding: 6.1 pmol/mg protein
K_d for [¹²⁵I]-SB258585 binding: ~0.44 nM

TRANSFECTION: Full-length human HTR6 cDNA encoding the 5-HT₆ Serotonin Receptor (Accession Number: NM_000871)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous 5-HT₆ 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: 20 mM HEPES, 3 mM MgCl₂, 2 mM Ascorbic acid, pH 7.4, filtered and stored at 4°C

Radioligand: [¹²⁵I]-SB258585 (Perkin Elmer #:NEX424)

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 6-fold signal:background with ¹²⁵I labeled SB258585 at 0.25 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 1 mg/mL in packaging buffer, and dispensed at 1 mL/vial. Vials were 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:

Woolley ML *et al.* (2004) 5-HT₆ receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3: 59-79.

Fisas A *et al.* (2006) Chronic 5-HT₆ receptor modulation by E-6837 induces hypophagia and sustained weight loss in diet-induced obese rats. *Br. J. Pharmacol.* 148: 973-83.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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