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PRODUCT DATASHEET

ChemiScreen™ 5-HT$_6$ Serotonin Membrane Preparation

CATALOG NUMBER:	HTS111M	**QUANTITY:**	200 units
LOT NUMBER:	2324477	**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-HT$_6$ is a Gs coupled receptor expressed solely in the CNS, primarily in the limbic and cortical regions. 5-HT$_6$ 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-HT$_6$-selective agonist caused significant weight loss in a rat model of diet-induced obesity. 5-HT$_6$ 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 Kd of 0.44 nM for [^{125}I]-SB258585. With 0.25nM [^{125}I]-SB258585, 5µg/well 5-HT$_6$ Membrane Prep typically yields greater than 6-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

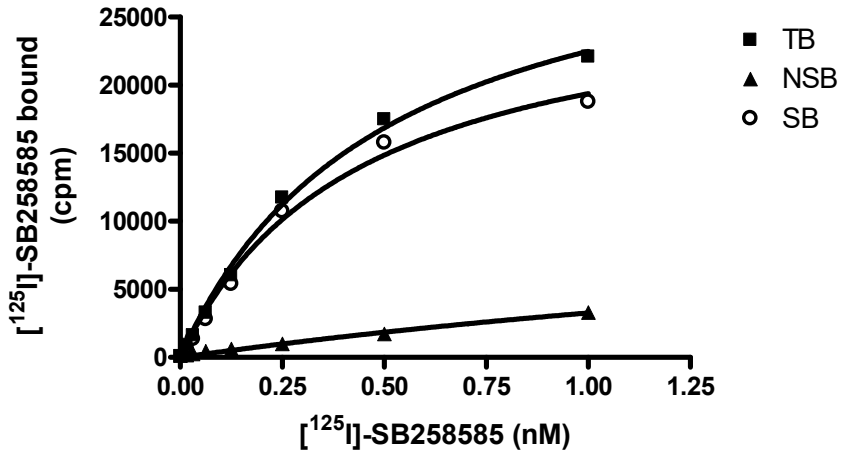

Figure 1. Saturation binding for 5-HT$_6$. 5 µg/well 5-HT$_6$ Membrane Preparation was incubated with increasing amount of ^{125}I-labeled SB-258585 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled 5-HT. Specific binding (SB) was determined by subtracting NSB from TB.

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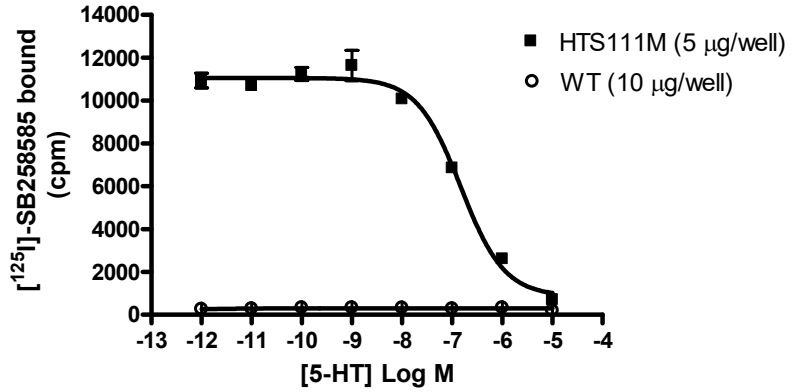

Figure 2. Competition binding for 5-HT$_6$. 5-HT$_6$ Membrane Preparation (5 µg/well) and wild-type Chem-1 Membrane Preparation (10 µg/well; Catalog # HTS000MC1) were incubated in a 96-well plate with 0.25 nM ^{125}I-labeled SB258585 and increasing concentrations of unlabeled 5-HT. More than 6-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
B_{max}: 6.1 pmol/mg protein
K_d: 0.44 nM
Signal:background: >6

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

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous 5-HT$_6$ 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.

Assay Buffer: 20 mM HEPES, 3 mM $MgCl_2$, 2 mM Ascorbic acid, pH 7.4, filtered and stored at 4°C

Radioligand: [^{125}I]-SB258585 (Perkin Elmer **#:**NEX424)

Wash Buffer: same as 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 ^{125}I 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: Membrane proteins were adjusted to the indicated concentration in 1 ml 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. Woolley ML *et al.* (2004) 5-HT_6 receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3: 59-79.

2. Fisas A *et al.* (2006) Chronic 5-HT_6 receptor modulation by E-6837 induces hypophagia and sustained weight loss in diet-induced obese rats. Br. J. Pharmacol. 148: 973-83.