

PRODUCT DATASHEET
ChemiScreen™ 5-HT_{2C} Serotonin Membrane Preparation

CATALOG NUMBER:	HTS132M	QUANTITY:	200 units
LOT NUMBER:	2055916	VOLUME/CONCENTRATION:	1 mL, 2 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). 5-HT_{2C}, which couples to G_q in most cells to stimulate intracellular calcium, is prominently expressed in brain and appears to modulate depression, anxiety and appetite (Miller, 2005; Serretti *et al.*, 2004; Wood, 2003). The mRNA encoding 5-HT_{2C} undergoes selective RNA editing that changes 4 amino acids in the second intracellular loop; these changes result in alteration of efficiency of coupling to G proteins. Alterations in editing of 5-HT_{2C} have been detected in victims of suicidal depression and in mice treated with the SSRI, fluoxetine (Tohda *et al.*, 2006). The 5-HT_{2C} 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_{2C} interactions and its ligands. The membrane preparations exhibit a K_d of 5.9 nM for [³H]-mesulergine. With 6 nM [³H]- Mesulergine, 10 µg/well 5-HT_{2C} Membrane Prep yields greater than 5 fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

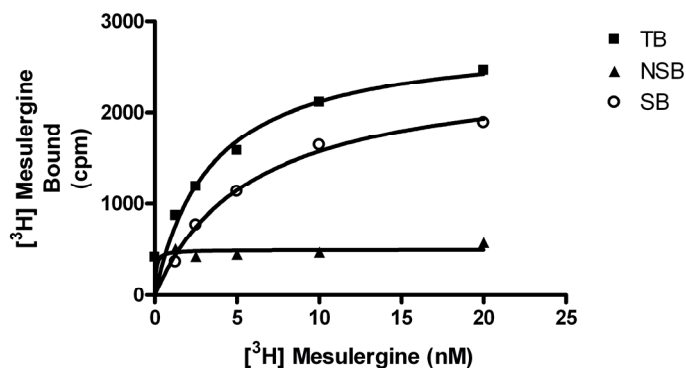


Figure 1. Saturation binding for 5-HT_{2C}. 5.0 µg/well 5-HT_{2C} Membrane Preparation was incubated with increasing amount of [³H]-Mesulergine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Ro 60-0175. Specific binding (SB) was determined by subtracting NSB from TB.

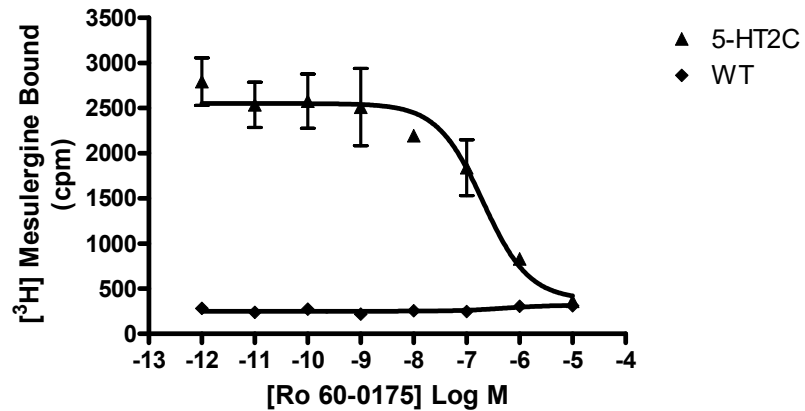


Figure 2. Competition binding for 5-HT_{2c}. 5-HT_{2c} Membrane Preparation and Wild-Type Chem-1 membrane preparation (each 10 mg/well) were incubated with 6.0 nM [³H]-Mesulergine and increasing concentrations of unlabeled Ro-60-0175, and more than 5-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
 B_{max}: 15.66 pmol/mg protein
 K_d: 5.9 nM
 Signal:background: >7.8

TRANSFECTION: 5-HT_{2c} (Accession number NM_000868)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous 5-HT_{2c} 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.

Assay Buffer: 50 mM Hepes, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, filtered and stored at 4°C

Radioligand: [³H]-Mesulergine (Amersham TRK 845)

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 5-fold signal:background with 3H-labeled Mesulergine at 6.0 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. Barnes NM and Sharp T (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38, 1083-1152.
2. Miller KJ (2005) Serotonin 5-HT_{2C} receptor agonists: potential for the treatment of obesity. *Mol. Interv.* 5: 282-91.
3. Serretti A *et al.* (2004) The 5-HT_{2C} receptor as a target for mood disorders. *Expert Opin. Ther. Targets* 8: 15-23.
4. Tohda M *et al.* (2006) The molecular pathopharmacology of 5-HT_{2C} receptors and the RNA editing in the brain. *J. Pharmacol. Sci.* 100: 427-432.
5. Wood MD (2003) Therapeutic potential of 5-HT_{2C} receptor antagonists in the treatment of anxiety disorders. *Curr. Drug Targets CNS Neurol. Disord.* 2: 383-7.

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