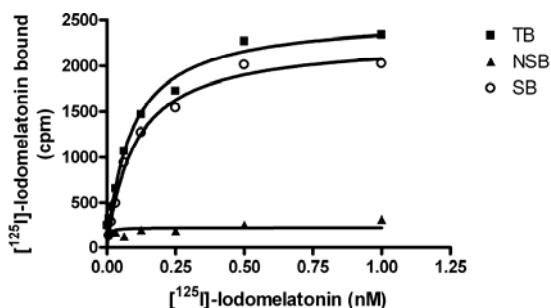


## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN MT<sub>2</sub> MELATONIN RECEPTOR

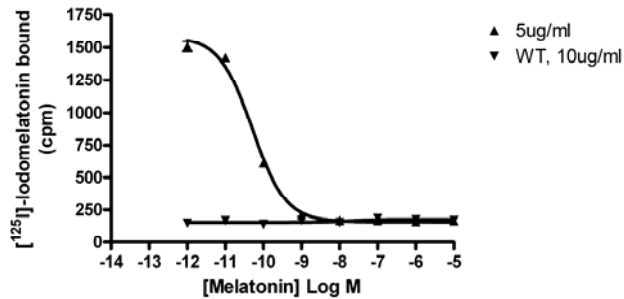
<b>CATALOG NUMBER:</b>	HTS106M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>		<b>VOLUME/CONCENTRATION PER VIAL:</b>	1 mL, 1 mg/mL

**BACKGROUND:** Melatonin (5-methoxy-acetyltryptamine) is a hormone synthesized and secreted in a circadian manner with high levels occurring in all species at night. Melatonin has been implicated in the regulation of various neural and endocrine processes that are cued by the daily change in photoperiod (Masana and Dubocovich, 2001). Two mammalian melatonin receptor subtypes, MT<sub>1</sub> and MT<sub>2</sub>, have been cloned. Both receptors are members of the superfamily of putative seven transmembrane G-protein coupled receptors, which are coupled to the inhibition of cyclic AMP synthesis upon agonist binding. Pharmacological studies suggest that the phase shifting effect of melatonin on circadian rhythms may be mediated by the MT<sub>2</sub> receptor subtype (Dubocovich *et al.* 1998). Melatonin inhibition of dopamine release from the retina is also mediated by the MT<sub>2</sub> receptor subtype (Dubocovich *et al.* 1997). Chemicon's MT<sub>2</sub> 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 at MT<sub>2</sub>. The membrane preparations exhibit a K<sub>d</sub> of 0.1 nM for [<sup>125</sup>I]-Iodomelatonin. With 0.2 nM [<sup>125</sup>I]-Iodomelatonin, 5 µg/well MT<sub>2</sub> Membrane Prep typically yields greater than 6-fold signal-to-background ratio.

**APPLICATIONS:** Radioligand binding assay and GTPγS binding.



**Figure 1. Saturation binding for MT<sub>2</sub>.** 5 µg/well MT<sub>2</sub> Membrane Preparation was incubated with increasing amount of <sup>125</sup>I-labeled Iodomelatonin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled melatonin. Specific binding (SB) was determined by subtracting NSB from TB.



**Figure 2. Competition binding for MT<sub>2</sub>.** 5 µg/well MT<sub>2</sub> Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 0.2 nM [<sup>125</sup>I]-labeled Iodomelatonin and increasing concentrations of unlabeled melatonin. More than 6- fold signal:background was obtained.

**Table 1.** Signal:background and specific binding values obtained in a competition binding assay with MT<sub>2</sub> membrane prep and unlabeled melatonin.

	5 µg/well
Signal:background	9.96
Specific binding (cpm)	1424.1

SPECIFICATIONS: 1 unit = 5 µg

B<sub>max</sub> for [<sup>125</sup>I]-Iodomelatonin binding: 0.04 pmol/mg protein

K<sub>d</sub> for [<sup>125</sup>I]-Iodomelatonin binding: ~0.1 nM

TRANSFECTION: Full-length human MTNR1B cDNA encoding MT<sub>2</sub> (Accession Number: NM\_005959)

HOST CELLS: Chem-1, an adherent mammalian cell line lacking endogenous MT<sub>2</sub> 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, 500mM NaCl. 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 MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, filtered and stored at 4°C

Radioligand: [<sup>125</sup>I] Iodomelatonin (Perkin Elmer#: NEX-236)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, filtered and stored at 4°C.

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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 <sup>125</sup>I-labeled Iodomelatonin at 0.2 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:**

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

**REFERENCES:**

Masana MI and Dubocovich ML (2001). Melatonin receptor signaling: finding the path through the dark. *Sci STKE*. Nov 6;2001(107):PE39.

Dubocovich ML, Yun K, Al-Ghoul WM, Benloucif S, Masana (1998). Selective MT2 melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. *FASEB J*. 12(12):1211-20.

Dubocovich ML, Masana MI, Iacob S, Sauri DM (1997) Melatonin receptor antagonists that differentiate between the human Mel1a and Mel1b recombinant subtypes are used to assess the pharmacological profile of the rabbit retina ML1 presynaptic heteroreceptor. *Naunyn Schmiedebergs Arch Pharmacol*. 355(3):365-75

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