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PRODUCT DATASHEET

ChemiScreen™ MCH1 Membrane Preparation

CATALOG NUMBER:	HTS025M	**QUANTITY:**	200 units
LOT NUMBER:	SC20161007	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

Melanin-concentrating hormone (MCH) is a cyclic, 19 amino acid peptide produced in the lateral hypothalamus and other regions of the mammalian brain (Bittencourt *et al.*, 1992). When administered intracerebrally or overexpressed transgenically in rodents, MCH stimulates food intake and weight gain (Ludwig *et al.*, 2001). A receptor for MCH, previously identified as an orphan receptor SLC-1 and renamed MCH_1, has been identified in humans and rodents. Consistent with a role in hypothalmic control of feeding behavior, MCH_1 is expressed in in the ventromedial and dorsomedial nuclei of the hypothalamus (Chambers *et al.*, 1999). Genetic ablation and pharmacological antagonism of MCH_1 in rodents results in resistance to obesity caused by a high fat diet or leptin deficiency (Borowsky *et al.*, 2002; Segal-Lieberman, *et al.*, 2003; Shearman *et al.*, 2003; Marsh *et al.*, 2002; Chen *et al.*, 2002). Thus, MCH_1 is an attractive target for obesity. MCH_1 membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level GPCR surface expression; thus, they are ideal HTS tools for screening for agonists and antagonists of MCH_1.

APPLICATIONS:

Radioligand binding assay

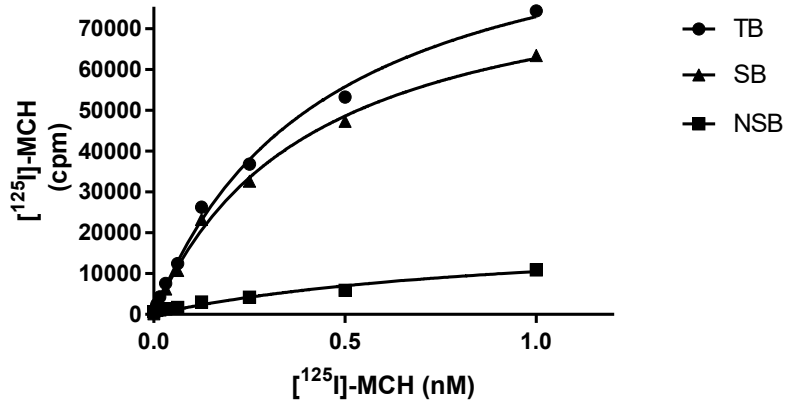

Figure 1. Saturation binding for MCH_1. 5.0 ug/well MCH_1 Membrane Preparation was incubated with increasing amount of [^{125}I]-MCH in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled MCH. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

Eurofins Pharma Bioanalytics Services US Inc.

15 Research Park Drive
St Charles MO 63304
USA

T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

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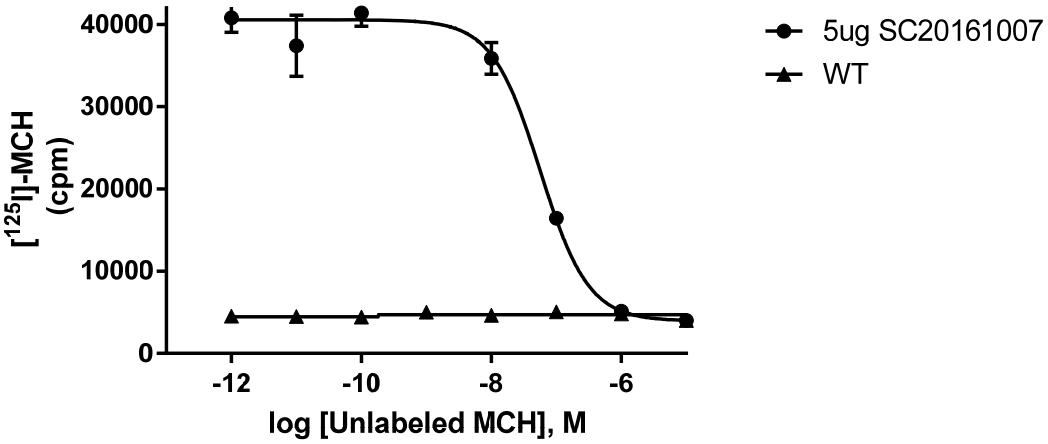

Figure 2. Competition binding for MCH_1. 5 ug/well MCH_1 Membrane Preparation and Wild-Type Chem-1 membrane preparation (10 µg/well; catalog # HTS000MC1) were incubated with 0.4 nM [^{125}I]-MCH and increasing concentrations of unlabeled MCH, and more than 8-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 195.96 pmol/mg protein
K_d: ~0.41 nM
Signal:background: >10-fold

TRANSFECTION: Full length human MCHR1 cDNA encoding MCH_1 (Accession number NM_005297)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable endogenous MCH1 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 (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. 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 $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{125}I]-MCH (Perkin Elmer CUSTOM)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4°C

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 8-fold signal:background with ^{125}I-labeled MCH at 0.4 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 -80ºC.

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:

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8. Shearman L.P., *et al.* (2003) Chronic MCH-1 receptor modulation alters appetite, body weight and adiposity in rats. *Eur. J. Pharmacol.* 475: 37-47.