

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN MC₅ MELANOCORTIN RECEPTOR

CATALOG NUMBER:	HTS155M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION PER VIAL:	2 mL, 1 mg/mL

BACKGROUND: The melanocortin system consists of five seven-transmembrane spanning G-protein coupled receptors (MC₁-MC₅). Among the five members of the melanocortin receptor family, MC₂ and MC₅ are expressed in peripheral tissues. The MC₂ receptor (ACTH receptor) is almost exclusively expressed in the adrenal cortex whereas MC₅ is expressed in several organs including the adrenal cortex. Studies have shown that targeted disruption of the MC5R gene produced mice with a severe defect in water repulsion and thermoregulation caused by decreased production of sebaceous lipids (Chen *et al.*, 1997). Data show a requirement for the MC₅ in multiple exocrine glands for the production of numerous products, indicative of a coordinated system for regulation of exocrine gland function by melanocortin peptides. (Entwistle *et al.*, 1990). Millipore's MC₅ 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 MC₅. The membrane preparations exhibit a K_d of 1.3nM for [¹²⁵I]-[Nle⁴, D-Phe⁷]-α-MSH (NDP-αMSH). With 0.3 nM [¹²⁵I]-NDP-αMSH, 10μg/well MC₅ Membrane Prep typically yields greater than 50-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay and GTPγS binding.

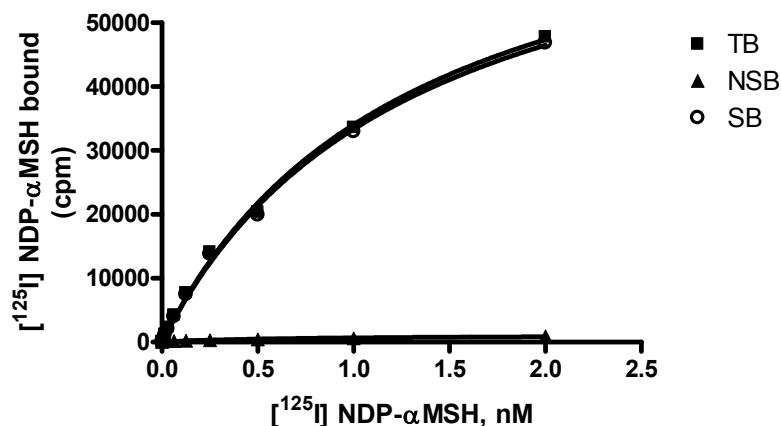


Figure 1. Saturation binding for MC₅. 5 μg/well MC₅ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-NDP-αMSH in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled NDP-αMSH. Specific binding (SB) was determined by subtracting NSB from TB.

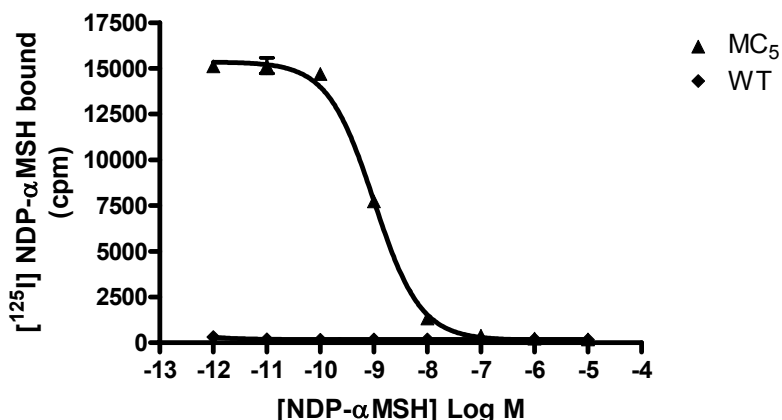


Figure 2. Competition binding for MC₅. MC₅ Membrane Preparation and Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1), each at 10 μg/well, were incubated with 0.3 nM [¹²⁵I]-NDP-αMSH and increasing concentrations of unlabeled NDP-αMSH, and more than 50- fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with MC₅ Receptor membrane prep.

	10 μg/well
Signal:background	147.3
Specific binding (cpm)	15208.7

SPECIFICATIONS: 1 unit = 10 μg

B_{max} for [¹²⁵I]-NDP-αMSH binding: 4.3 pmol/mg protein

K_d for [¹²⁵I]-NDP-αMSH binding: ~1.3 nM

TRANSFECTION: Full-length human MC5R cDNA encoding MC₅ (Accession Number: NM_005913)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous MC₅ 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 MgCl₂, 1 mM CaCl₂, filtered and stored at 4°C.

Radioligand: [¹²⁵I]-NDP-αMSH (Perkin Elmer#: NEX-352)

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

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 50-fold signal:background with ¹²⁵I labeled NDP- α MSH at 0.3 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 the indicated concentration 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:

Chen W *et al.* (1997) Exocrine gland dysfunction in MC5-R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides. *Cell* 91: 789-798.

Entwistle ML *et al.* (1990) Characterization of functional melanotropin receptors in lacrimal glands of the rat. *Peptides* 11: 477-483.

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