PRODUCT DATASHEET

ChemiScreen™ MC_5 Melanocortin Membrane Preparation

CATALOG NUMBER:	HTS155M	**QUANTITY:**	200 units
LOT NUMBER:	SC20190807	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: The melanocortin system consists of five seven-transmembrane spanning G-protein coupled receptors (MC_1-MC_5). Among the five members of the melanocortin receptor family, MC_2 and MC_5 are expressed in peripheral tissues. The MC_2 receptor (ACTH receptor) is almost exclusively expressed in the adrenal cortex whereas MC_5 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 MC_5 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). MC_5 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 MC_5.

APPLICATIONS: Radioligand Binding Assay

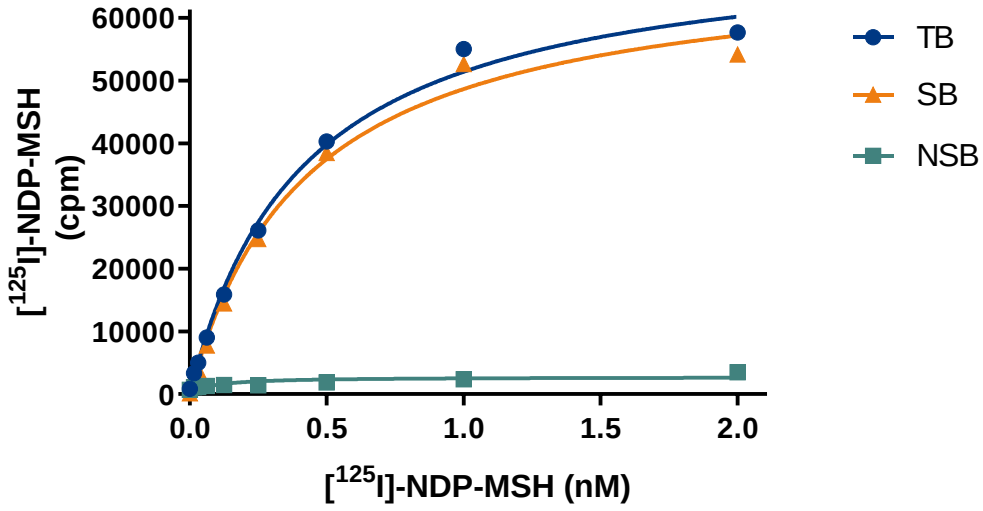

Figure 1. Saturation Binding for MC_5. 10 µg/well MC_5 Membrane Preparations were incubated with increasing amount of [^{125}I]-NDP-αMSH in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled NDP-αMSH. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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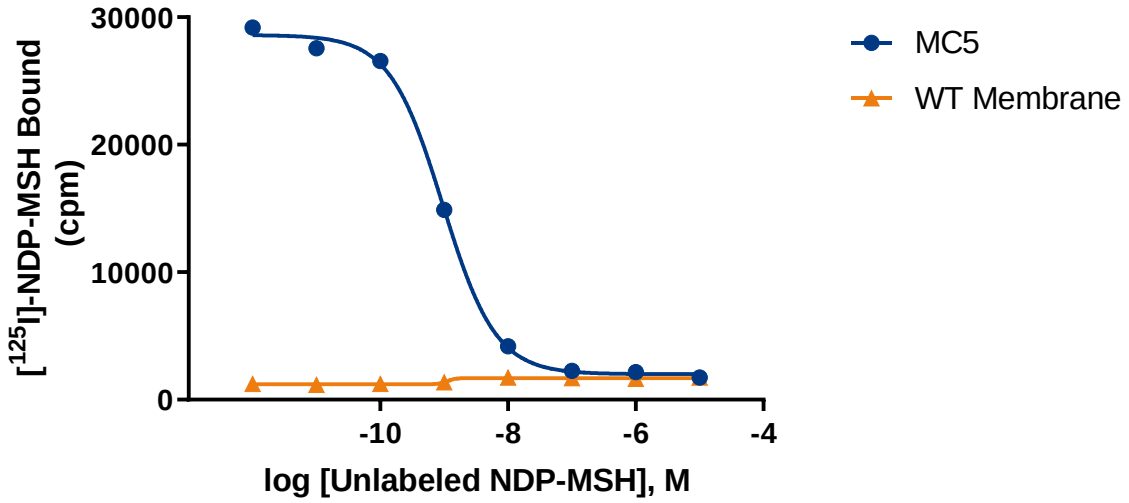

Figure 2. Competition Binding for MC_5. MC_5 Membrane Preparation and Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1), each at 10 µg/well, were incubated with 0.3 nM [^{125}I]-NDP-αMSH and increasing concentrations of unlabeled NDP-αMSH, and more than 15- fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{125}I]-NDP-αMSH binding: 2.84 pmol/mg
K_d for [^{125}I]-NDP-αMSH binding: 0.43 nM
Signal:background: ≥15-fold

TRANSFECTION: Full-length human MC5R cDNA encoding MC_5 (Accession Number: NM_005913).

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous MC_5 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 50 mM HEPES, pH 7.4, 500 mM 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 $MgCl_2$, 1 mM $CaCl_2$, filtered and stored at 4°C.

Radioligand: [^{125}I]-NDP-αMSH (PerkinElmer#**:** NEX-352)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM 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 [^{125}I] NDP-αMSH 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: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80^{o}C.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. 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.

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

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