CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN MC_4 MELANOCORTIN RECEPTOR

CATALOG NUMBER: HTS105M **QUANTITY:** 200 units

LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

BACKGROUND:

The melanocortins, α-, β- and γ-melanocyte-stimulating hormones (MSHs), are peptides derived from a precursor protein POMC. The MSH peptides bind to a family of five G_s-coupled seven transmembrane recptors (MCRs) and play important roles in energy balance, reproductive function, pigmentation and inflammation (Gantz and Fong, 2003). The activity of the melanocortins is modulated by endogenous antagonist proteins, agouti and agouti-related protein (AGRP). MC4R is expressed primarily in the CNS and appears to play a prominent role in energy homeostasis. The hypothalamus, which is a major central site for control of feeding behavior, prominently expresses MC4R. Targeted deletion of MC4R in mice and naturally occurring mutations in MC4R in humans result in obese phenotypes. In addition, blockade of MC4R function by antagonists and targeted deletion of the gene in mice reverses melanocortin agonist-induced inhibition of food intake and promotes weight gain in uremia and cancer-induced cachexia (Cheung *et al*., 2005; Markison *et al*., 2005). Chemicon's MC_4 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 MC_4 interactions with αMSH. The membrane preparations exhibit a Kd of 0.71-0.77 nM for [^{125}I]-NDP-αMSH. With 5 µg/well MC_4 Membrane Prep and 0.5 nM [^{125}I]-NDP-αMSH, a greater than 15-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

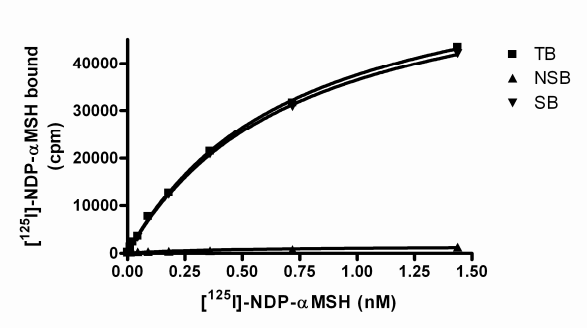

Figure 1. Saturation binding for MC_4. 5 µg/well MC_4 Membrane Preparation was incubated with increasing amount of [^{125}I]-NDP-αMSH in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 5000-fold excess unlabeled NDP-MSH. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

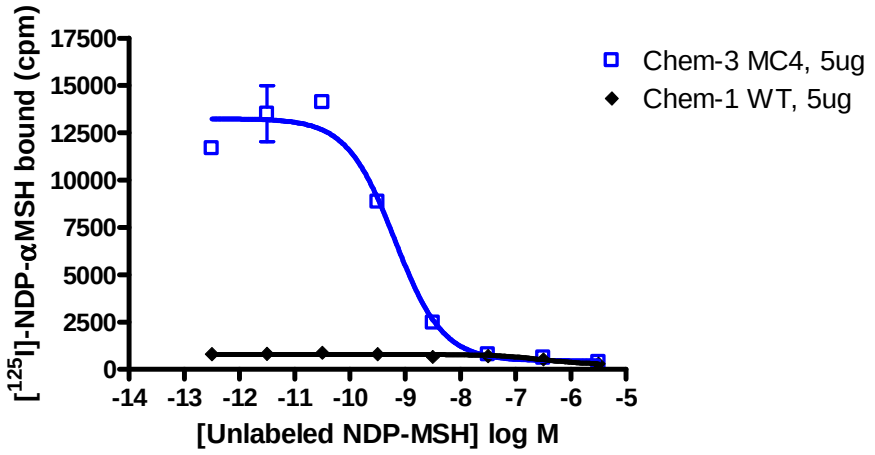

Figure 2. Competition binding for MC_4. MC_4 Membrane Preparation (5 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.5 nM [^{125}I]-NDP-αMSH and increasing concentrations of unlabeled NDP-MSH, and more than 15- fold signal:background was obtained. Representative sample data.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with EP_1 membrane prep.

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
Bmax: 5.5 pmol/mg
K_d: 0.74 nM
Signal:background: >15 fold

Species: Full-length human MC4R cDNA encoding for MC_4 gene (Accession Number: NM_005912)

HOST CELLS: Chem-3, a suspension mammalian cell line without any endogenous MC_4 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, a GF/C 96-well filter plate 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℃

Radioligand: [^{125}I] NDP-αMSH (Perkin Elmer # NEX352)
Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4℃.

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 15-fold signal:background with ^{125}I-labeled NDP-αMSH at 0.5 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, rapidly frozen, and stored at -80^{o}C.

STORAGE/HANDLING: Store at –70℃. Product is stable for at least 6 m onths from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

Cheung W *et al.* (2005) Role of leptin and melanocortin signaling in uremia-associated cachexia. *J. Clin. Invest.* 115: 1659-1665.

Gantz I and Fong TM (2003) The melanocortin system. *Am. J. Physiol. Endocrinol. Metab.* 284: E468-E474.

Markison S *et al.* (2005) The regulation of feeding and metabolic rate and the prevention of murine cancer cachexia with a small-molecule melanocortin-4 receptor antagonist. *Endocrinology* 146: 2766-2773.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION