

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN MC₁ MELANOCORTIN RECEPTOR

CATALOG NUMBER:	HTS156M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION PER VIAL:	1 mL, 1 mg/mL

BACKGROUND: The melanocortin-1 receptor (MC₁) is a member of the G-protein-coupled receptor family of proteins that is responsible for regulating skin and hair color. The MC₁ receptor is regulated by the physiological agonists alpha-melanocyte-stimulating hormone (alphaMSH) and adrenocorticotropin hormone (ACTH), and antagonist agouti signaling protein (ASP) (Rouzud *et al.*, 2005). In 1995 a landmark study demonstrated that over 80% of humans with red hair or fair skin have a dysfunctional variant of the MC₁ gene (Valverde *et al.*, 1995). MC₁ is, therefore, a major determinant of sun sensitivity and genetic risk factor for melanoma and non-melanoma skin cancer. Millipore's MC₁ 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 MC₁. 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, 5μg/well MC₁ Membrane Prep typically yields greater than 50-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay and GTPγS binding.

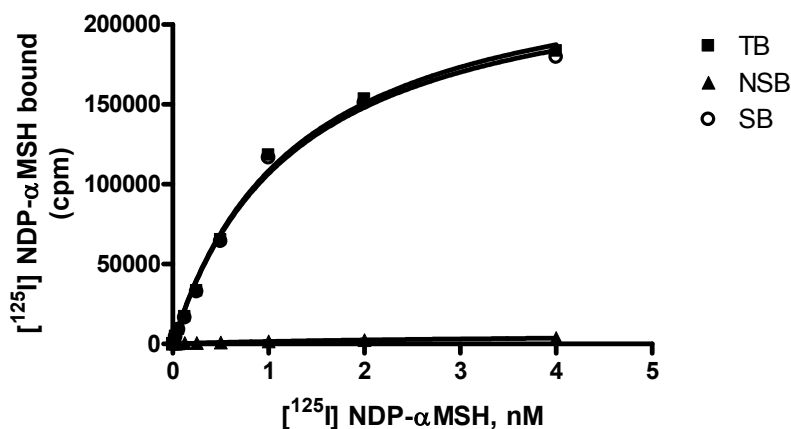


Figure 1. Saturation binding for MC₁. 5 μg/well MC₁ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-NDP-αMSH in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled NDP-αMSH. Specific binding (SB) was determined by subtracting NSB from TB.

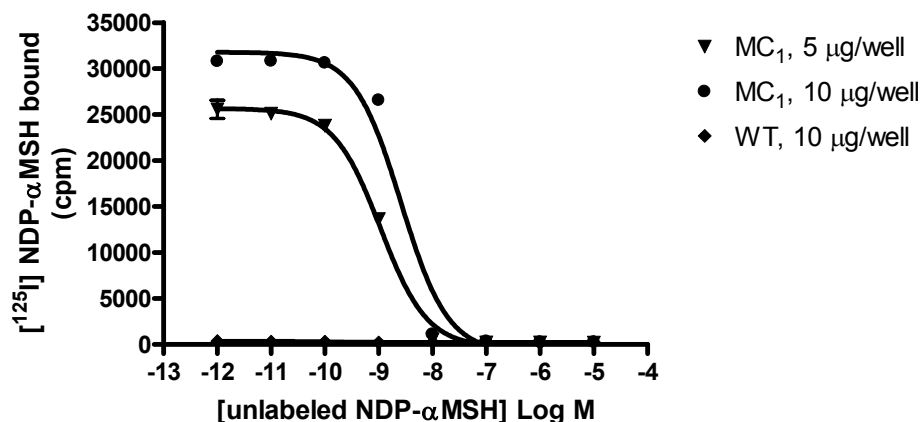


Figure 2. Competition binding for MC₁. MC₁ Membrane Preparation (5 or 10 μg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.3 nM [¹²⁵I]-NDP-αMSH and increasing concentrations of unlabeled NDP-αMSH, and more than 50- fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with MC₁Receptor membrane prep.

	5 μg/well	10 μg/well
Signal:background	146.6	133
Specific binding (cpm)	25485	31544

SPECIFICATIONS: 1 unit = 5 μg
 B_{max} for [¹²⁵I]-NDP-αMSH binding: 13.7 pmol/mg protein
 K_d for [¹²⁵I]-NDP-αMSH binding: ~1.3 nM

TRANSFECTION: Full-length human MC1R cDNA encoding MC₁ (Accession Number: NM_002386)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous MC₁ 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 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:

Valverde P *et al.* (1995) Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat. Genet.* 11: 328-30.

Rouzaud F *et al.* (2005) MC1R and the response of melanocytes to ultraviolet radiation. *Mutat. Res.* 571: 133-152.

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