

PRODUCT DATASHEET
ChemiScreen™ MC₃ Melanocortin Membrane Preparation

CATALOG NUMBER:	HTS022M	QUANTITY:	200 units
LOT NUMBER:	2349730	VOLUME/CONCENTRATION:	1 mL, 2 mg/mL

BACKGROUND: The melanocortins, the peptides α -, β - and γ -melanocyte-stimulating hormone (MSH), bind to a family of five Gs-coupled GPCR receptors (MC₁₋₅) and play important roles in energy balance, reproductive function, pigmentation and inflammation (Gantz and Fong, 2003). MC₃ is expressed in the CNS, including the hypothalamus, and appears to play a role in energy homeostasis as indicated by the observation that MC₃-null mice have increased subcutaneous fat, a loss of lean body mass, and reduced energy expenditure (Butler et al., 2000; Chen et al., 2000). Macrophages also express MC₃, which together with MC₁ mediates the anti-inflammatory activity of melanocortins in animal models of peritonitis and myocardial infarct (Getting et al., 2003; 2004). MC₃ 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 MC₃ interactions with melanocortins. The membrane preparations exhibit a K_d of 0.4 nM for [¹²⁵I]-MSH. With 10.0 μ g/well MC₃ Membrane Prep and 0.25 nM [¹²⁵I]-MSH analog, a greater than 4-fold signal-to-background ratio is obtained.

APPLICATIONS: Radioligand binding assay and GTP γ S binding

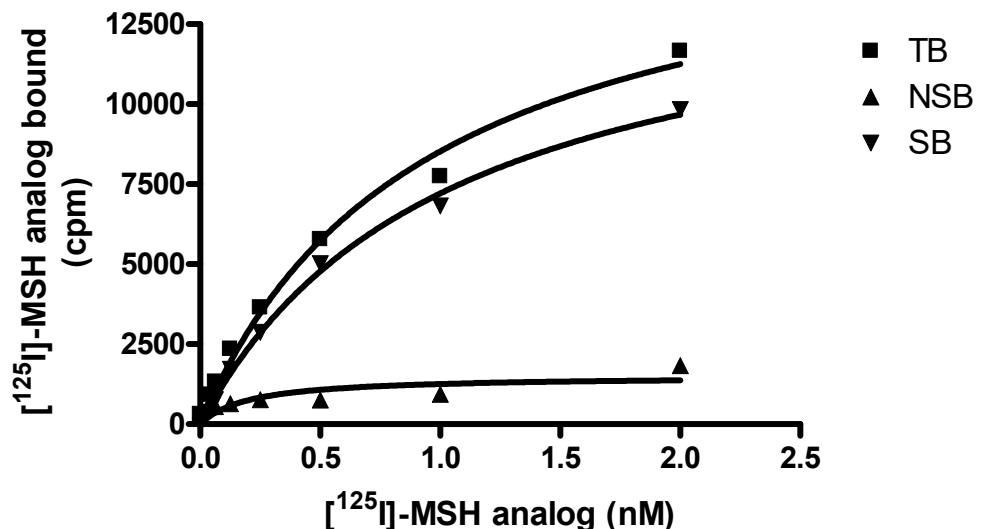


Figure 1. Saturation binding for MC₃. 10 μ g/well MC₃ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-MSH analog in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled MSH analog, and subjected to filtration binding as described in "Recommended Assay Conditions" below. Specific binding (SB) was determined by subtracting NSB from TB.

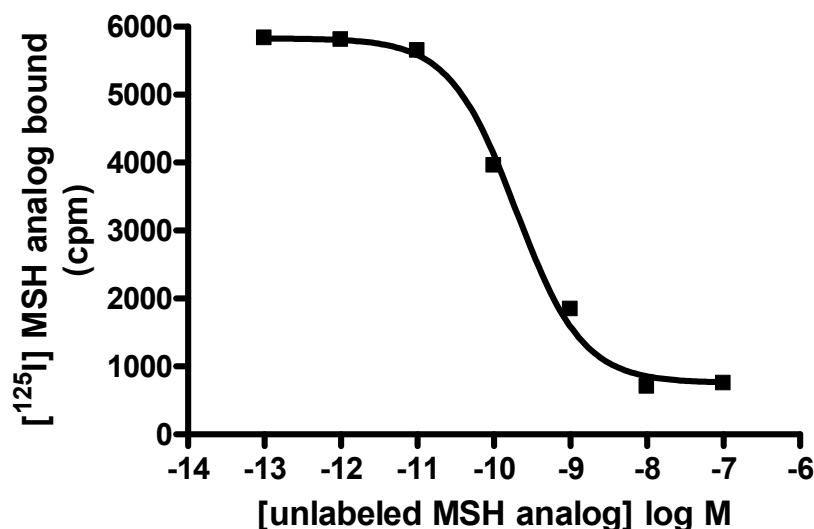


Figure 2. Competition binding for MC₃. 10 µg/well MC₃ Membrane Preparation (HTS022M) was incubated with 0.25 nM [¹²⁵I]-MSH analog and increasing concentrations of unlabeled MSH analog, and subjected to filtration binding as described in "Recommended Assay Conditions" below. More than 4- fold signal: background was obtained.

SPECIFICATIONS: 1 unit = 10 µg
 B_{max} for [¹²⁵I]- [125I]-MSH analog binding: 0.77 pmol/mg protein
 K_d for [¹²⁵I]- [125I]-MSH analog binding: ~1.0 nM
 Signal:background: >4-fold

TRANSFECTION: Full-length human MC₃R cDNA encoding MC₃ (Accession number L06155)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous MC₃ 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, 0.5% BSA. Binding reaction is transferred to the filter plate, and washed 9 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 25 mM HEPES, pH 7.0, 1 mM MgCl₂, 1.5 mM CaCl₂ 2H₂O, 100mM NaCl, 0.2% BSA, Protease Inhibitor, filter and stored at 4°C.

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

Wash Buffer: 25 mM HEPES, pH 7.0, 1 mM MgCl₂, 1.5 mM CaCl₂ 2H₂O, 100mM NaCl, 0.2% BSA, 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 4-fold signal:background with ¹²⁵I labeled NDP-αMSH at 0.25 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:** 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:**
1. Chen AS *et al.* (2000) Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat. Genet.* 26: 97-102.
 2. Gantz I and Fong TM (2003) The melanocortin system. *Am. J. Physiol. Endocrinol. Metab.* 284: E468-E474.

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