

human Melanocortin MC₄ Receptor

Product Number: RBHMC4M400UA

Lot Number: 2937889

Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background: HEK293

GenBank Accession Number: NM_005912

Unit Size: 2 µg protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl₂, 10% sucrose.

Storage Conditions: Store at -80°C. Freeze-thaw is not recommended as it can affect product performance and homogeneity. In order to minimize negative impact of freeze-thawing, flash freeze in liquid nitrogen for 30 seconds prior to transferring to -80°C.

Stability: This product is stable for at least 3 years from reception if used and stored under recommended conditions.

Quality Control

B_{max} and K_d are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method ⁽¹⁾. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot (B_{max} in fmoles) by the maximal signal of a pre-defined reference tested in parallel. RTR is an indicator of lot-to-lot consistency. *We certify that these results meet our quality release criteria.

Ratio-to-Reference (RTR): N/A

Expression Level (B_{MAX}): 9.8 pmol/mg membrane protein.

K_D for [¹²⁵I]-(Nle⁴, D-Phe⁷)-α-MSH : 0.18 nM

Protein Concentration: 2 µg/µL

(1) Smith, P.K., et al. (1985). *Anal. Biochem.* 150, 76-85.

Recommended Assay Conditions

Assay Buffer: 25 mM Hepes pH 7.0, 1.5 mM CaCl₂, 1 mM MgSO₄, 100 mM NaCl, 0.2% BSA, 1 mM 1,10-phenanthroline, 1 Complete™ protease inhibitor tablet (EDTA free)/100 mL

Wash Buffer: 25 mM Hepes pH 7.0, 1.5 mM CaCl₂, 1 mM MgSO₄, 100 mM NaCl

Binding Protocol: Binding assays are performed in 200 µL total volume according to the following conditions:

1 - Membrane dilution: 0.05 mL of membranes + 7.45 mL assay buffer (1:150 dilution)

2 - Incubation: 25 µL of incubation buffer or (Nle⁴,D-Phe⁷)-α-MSH (Bachem H-1100) 3 µM final for non specific binding (Saturation binding assay)

For competition binding assay: 25 µL of reference compounds at decreasing concentrations (see figure 2)

25 µL of radioligand at the appropriate concentration (see graph below)
150 µL of diluted membranes

3 - Incubation time: 120 minutes at 37 °C

4 - Filtration: aspirate and wash 9 x 500 µL with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

Lot Specific Data

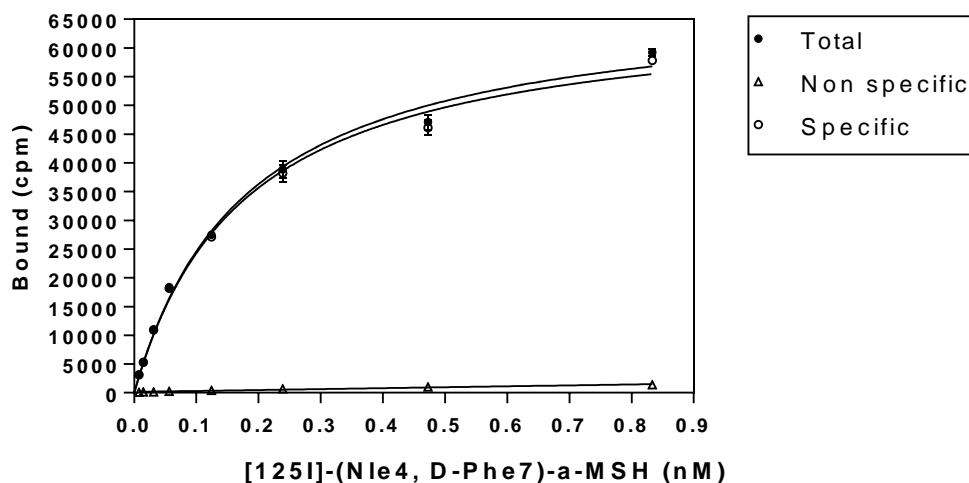


Figure 1: Saturation binding assay curve (filtration) 96-well saturation binding assay curve (2 µg membranes/well, TopCount®) using [¹²⁵I]-(Nle⁴, D-Phe⁷)-α-MSH (Revvity NEX352 Lot No.: IMB2410)

Typical Product Data

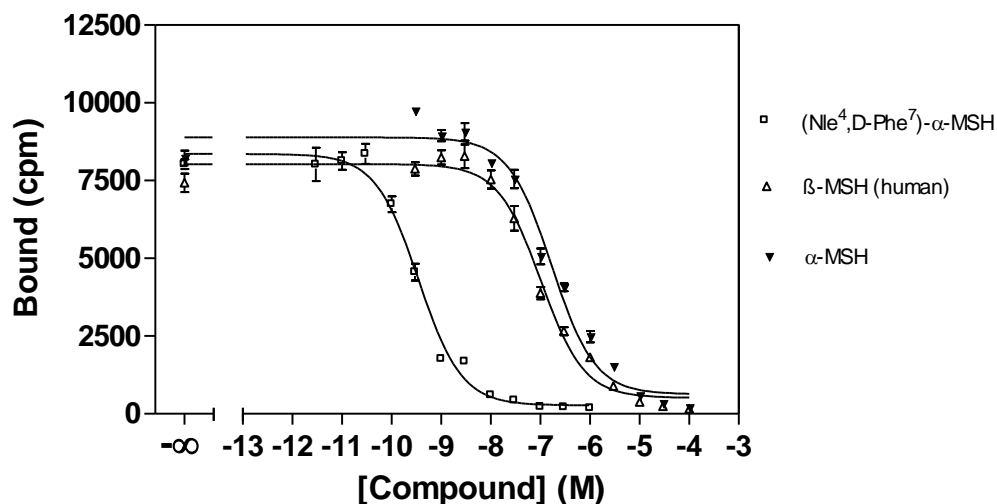


Figure 2: Competition binding assay curve (filtration)
 96-well competition binding assay curve (2 μ g membranes/well, TopCount®). Recommended radioligand concentration = 0.2 nM.

*Even though two sites can be observed occasionally with some ligands, the data presented is derived from single site fitting.

Reference Compounds	Ki (nM)
(Nle ⁴ ,D-Phe ⁷)- α -MSH	0.22
β -MSH (human)	65
α -MSH	107

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