

## human Melanocortin MC<sub>3</sub> Receptor

Product No.: ES-193-M400UA

Lot No.: 2198423

### Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

### Product Information

Cellular Background: CHO-K1

GenBank Accession Number: NM\_019888 (T6K)

Unit Size: 8 µg protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl<sub>2</sub>, 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<sub>max</sub> and K<sub>d</sub> are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method <sup>(1)</sup>. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot (B<sub>max</sub> 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): 1.5

Expression Level (B<sub>max</sub>): 2.8 pmol/mg membrane protein.

K<sub>d</sub> for [<sup>125</sup>I]-(Nle<sup>4</sup>, D-Phe<sup>7</sup>)-α-MSH: 0.22 nM

Protein Concentration: 8 µg/µL

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

## Recommended Assay Conditions

Assay Buffer:	25 mM Hepes pH 7.4, 10 mM MgCl <sub>2</sub> , 1 mM CaCl <sub>2</sub> , 0.5% BSA
Wash Buffer:	25 mM Hepes pH 7.4, 5 mM MgCl <sub>2</sub> , 1 mM CaCl <sub>2</sub> , 500 mM NaCl
Binding Protocol:	Binding assays are performed in 200 $\mu$ L 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 $\mu$ L of incubation buffer or (Nle <sup>4</sup> ,D-Phe <sup>7</sup> )- $\alpha$ -MSH (Bachem H-1100) 30 $\mu$ M final for non-specific binding (Saturation binding assay)
	<i>For competition binding assay: 25 <math>\mu</math>L of reference compounds at decreasing concentrations (see figure 2)</i>
	25 $\mu$ L of radioligand at the appropriate concentration (see graph below) 150 $\mu$ L of diluted membranes
3 - Incubation time:	60 minutes at 27 $^{\circ}$ C
4 - Filtration:	aspirate and wash 9 x 500 $\mu$ L with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

## Lot Specific Data

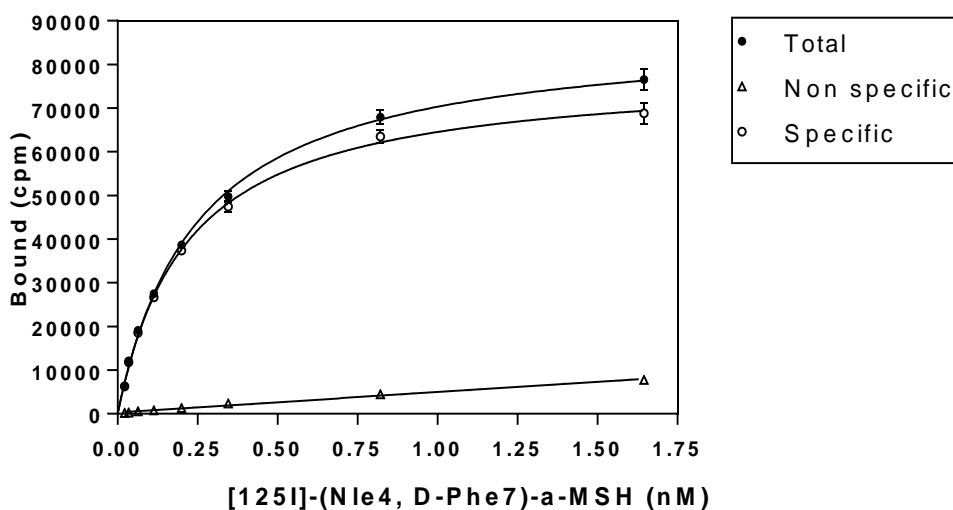


Figure 1: Saturation binding assay curve (filtration)  
96-well saturation binding assay curve (8  $\mu$ g membranes/well, TopCount<sup>®</sup>) using [<sup>125</sup>I]-(Nle<sup>4</sup>, D-Phe<sup>7</sup>)- $\alpha$ -MSH (PerkinElmer NEX352 Lot No.: IM02160)

Typical Product Data

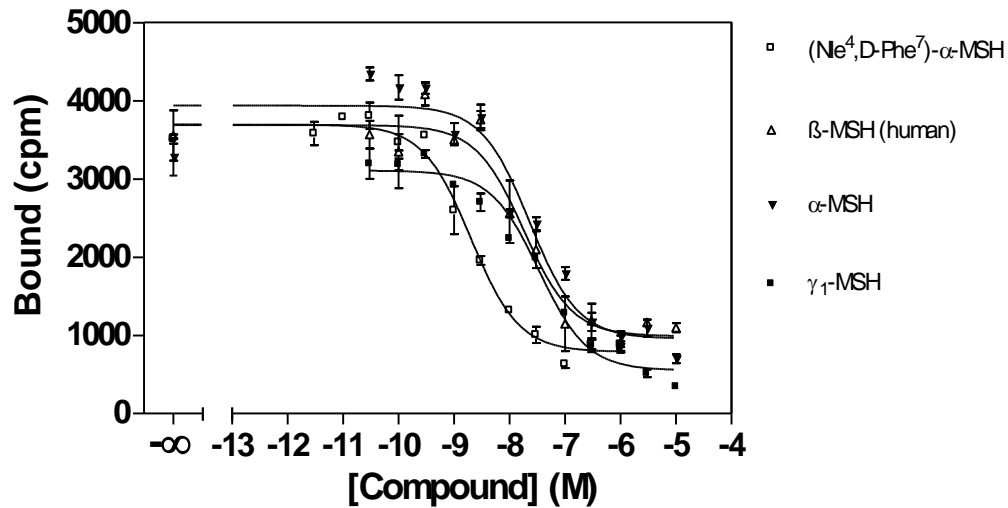


Figure 2: Competition binding assay curve (filtration)  
96-well competition binding assay curve (8  $\mu$ g membranes/well, TopCount®). Recommended radioligand concentration = 0.15 nM.

\*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 <sup>4</sup> ,D-Phe <sup>7</sup> )- $\alpha$ -MSH	1.8
$\beta$ -MSH (human)	17
$\alpha$ -MSH	21
$\gamma_1$ -MSH	32

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