human Melanocortin MC_4 Receptor

Product No.: ES-191-M400UA

Lot No.: 2281322

Material Provided

Membranes:	1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background:	CHO-K1
GenBank Accession Number:	L08603 (I103V)
Unit Size:	1.5 µg protein / unit
Storage Buffer:	50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM $MgCl_2$, 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):	0.5
Expression Level (B_{max}):	40 pmol/mg membrane protein.
K_d for [^{125}I]-(Nle^4, D-Phe^7)-α-MSH:	0.25 nM
Protein Concentration:	1.5 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 mM $MgSO_4$, 1 mM $CaCl_2$, 0.2% BSA, 1 mM 1,10 Phenantroline, 2 Complete™ protease inhibitor tablet (EDTA free)/100 mL
Wash Buffer:	50 mM Tris-HCl pH 7.4
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 (Nle^4,D-Phe^7)-α-MSH (Bachem H-1100) 10 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 27 °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

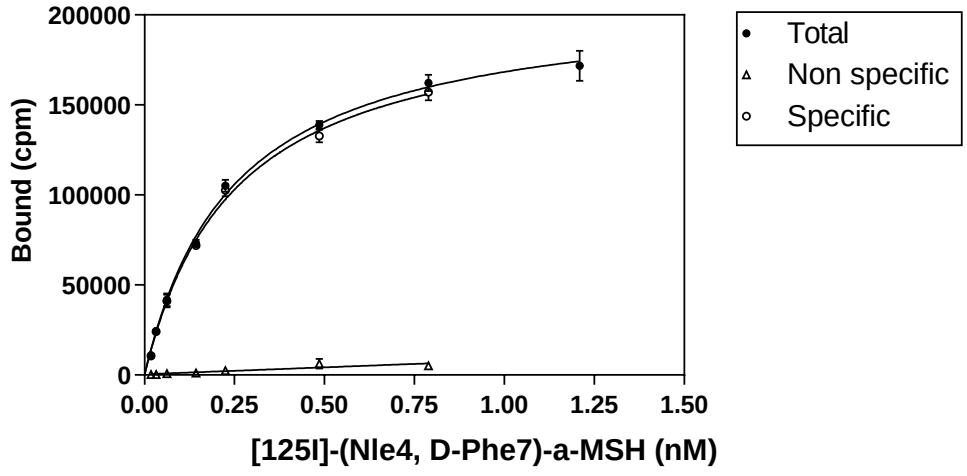

Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (1.5 µg membranes/well, TopCount®) using [^{125}I]-(Nle^4, D-Phe^7)-α-MSH (Revvity NEX352 Lot No.: IM62370)

Typical Product Data

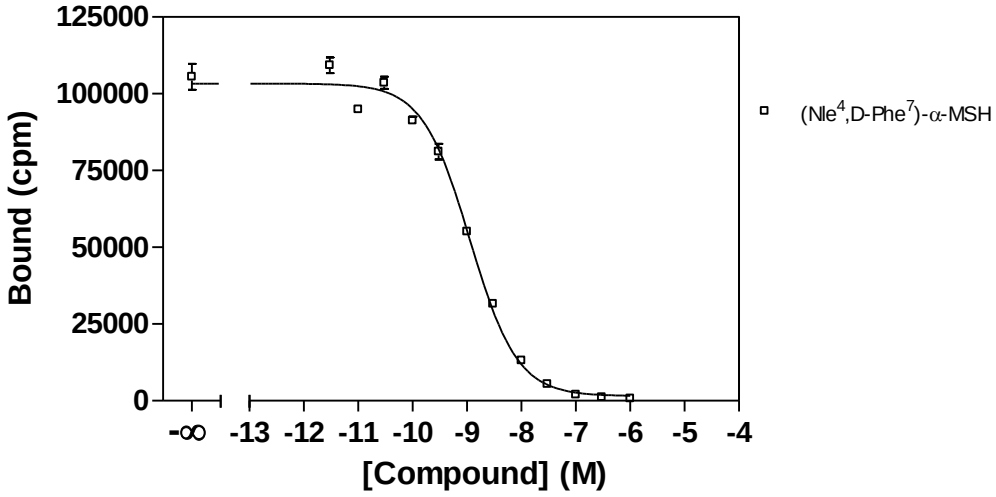

Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (1.5 µg membranes/well, TopCount®). Recommended radioligand concentration = 0.5 nM.

*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	0.67

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