human Melanocortin MC_3 Receptor

Product Number: RBXMC3M400UA

Lot Number: 2399282

Material Provided

Membranes:	1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background:	HEK293
GenBank Accession Number:	NM_019888 (T6K, V81I, G360A)
Unit Size:	4.8 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

Bmax and Kd 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 (Bmax 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):	0.52
Expression Level (B_{MAX}):	2.5 pmol/mg membrane protein.
K_D for [^{125}I]-(Nle^4, D-Phe^7)-α-MSH:	0.13 nM
Protein Concentration:	4.8 µg/µL

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

Recommended Assay Conditions

Assay Buffer:	25 mM Hepes pH 7.0, 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 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 $CaCl_2$, 1 mM $MgSO_4$, 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 (Nle^4,D-Phe^7)-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:	60 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

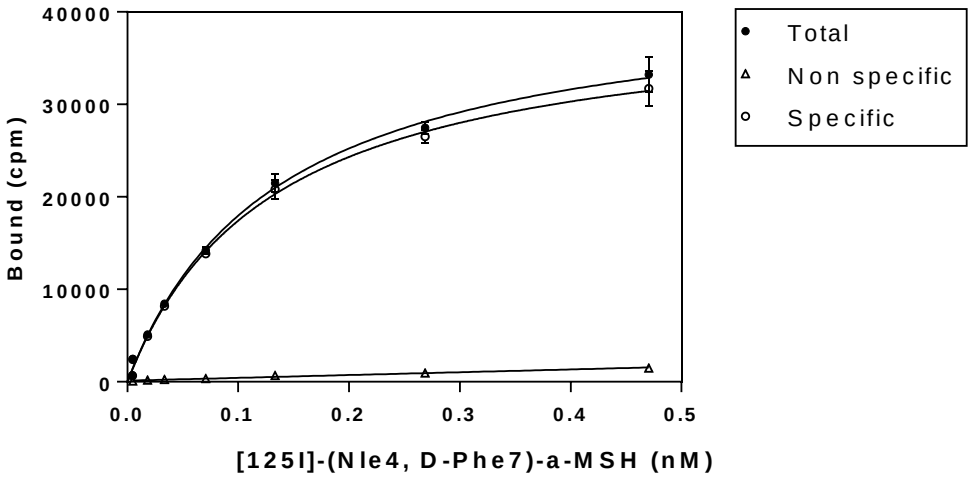

Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (4.8 µg membranes/well, TopCount®) using [^{125}I]-(Nle^4, D-Phe^7)-α-MSH (Revvity NEX352 Lot No.: IM62280)

Typical Product Data

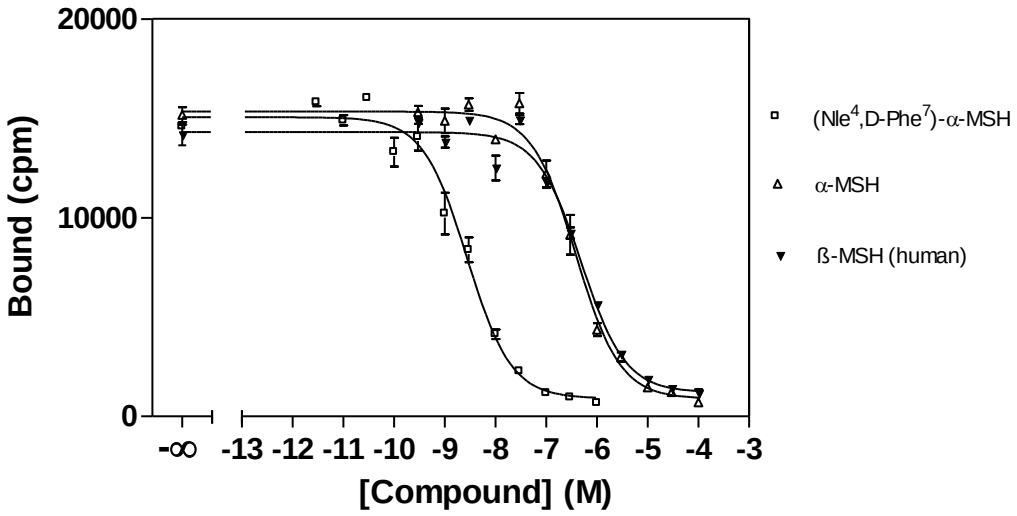

Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (4.8 µg membranes/well, TopCount®). Recommended radioligand concentration = 0.8 nM.

*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	0.99
α-MSH	139
ß-MSH (human)	176

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