

Caution: For Laboratory Use. A research reagent for research purposes only

human Ghrelin Receptor

Product No.: RBHGSM400UA

Lot No.: 492-254-A

Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background: HEK293

GenBank Accession Number: NM_198407

Unit Size: 0.8 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}): 1.0 pmol/mg membrane protein.

K_D for [¹²⁵I]-Ghrelin : 0.022 nM

Protein Concentration: 0.8 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, 1 mM CaCl₂, 5mM MgCl₂, 2.5 mM EDTA, 0.4% BSA

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 Ghrelin (human) (Bachem H-4864) 1 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 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

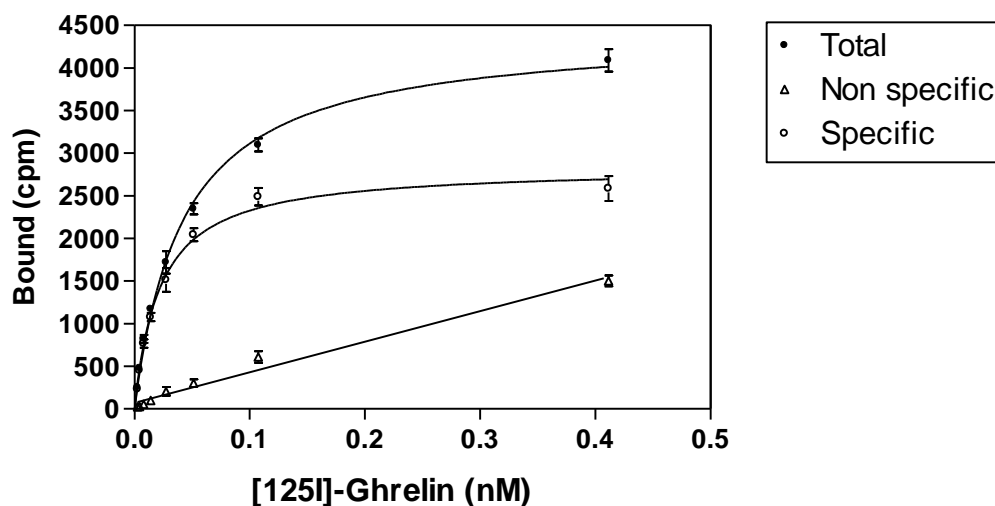


Figure 1: Saturation binding assay curve (filtration)

96-well saturation binding assay curve (0.8 µg membranes/well, TopCount®) using [¹²⁵I]-Ghrelin (PerkinElmer NEX388 Lot No.: JW62780)

Typical Product Data

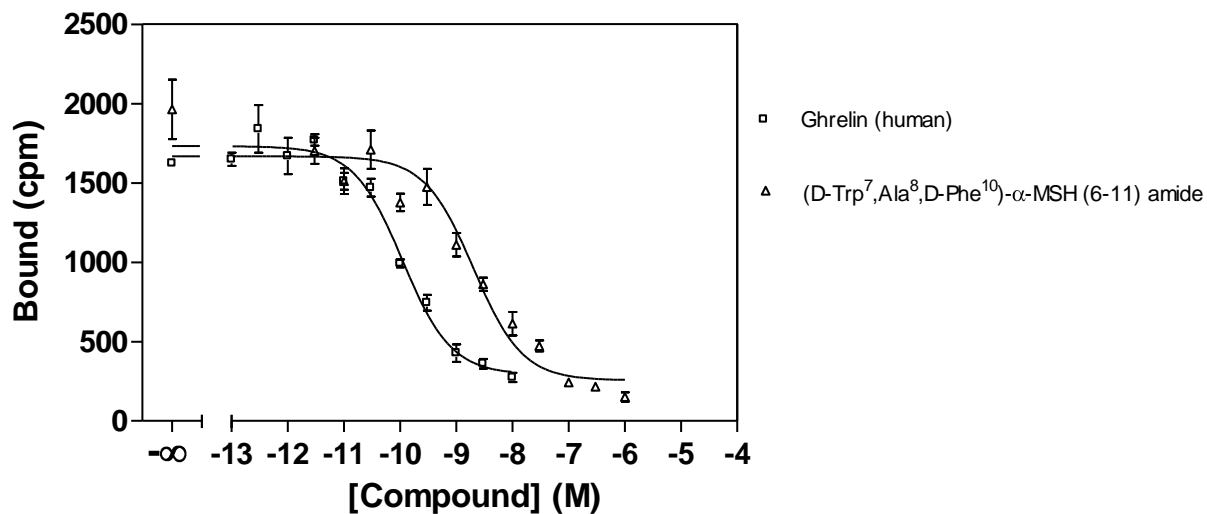


Figure 2: Competition binding assay curve (filtration)

96-well competition binding assay curve (0.8 µg membranes/well, TopCount®). Recommended radioligand concentration = 0.033 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)
Ghrelin (human)	0.050
(D-Trp ⁷ ,Ala ⁸ ,D-Phe ¹⁰)-α-MSH (6-11) amide	0.93

Suggested Materials and Instrumentation

Please visit our website

www.perkinelmer.com/GPCR

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