

# Technical Data Certificate of Analysis

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

## Membrane Target Systems™

### human Ghrelin Receptor

PRODUCT NO.: RBHGHSM400UA

LOT NO.: 462-028-A

#### MATERIAL PROVIDED

**MEMBRANES:** 1 x 400 units / 400 µl frozen aliquot.

**PACKAGING BUFFER:** 50 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 10 mM MgCl<sub>2</sub>, 10% sucrose.

#### PRODUCT INFORMATION

**CELLULAR BACKGROUND:** HEK293

**PROTEIN ACCESSION NUMBER:** NM\_198407

**UNIT SIZE:** 0.8 µg protein / unit

**STORAGE CONDITIONS:** Store at -80°C. Avoid repeated freeze-thaw.

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

#### QUALITY CONTROL

**EXPRESSION LEVEL (B<sub>Max</sub>):** 1.04 pmol/mg membrane protein.

**K<sub>D</sub> FOR [<sup>125</sup>I]-GHRELIN :** 0.022 nM

**PROTEIN CONCENTRATION:** 0.8 mg/ml

#### RECOMMENDED ASSAY CONDITIONS

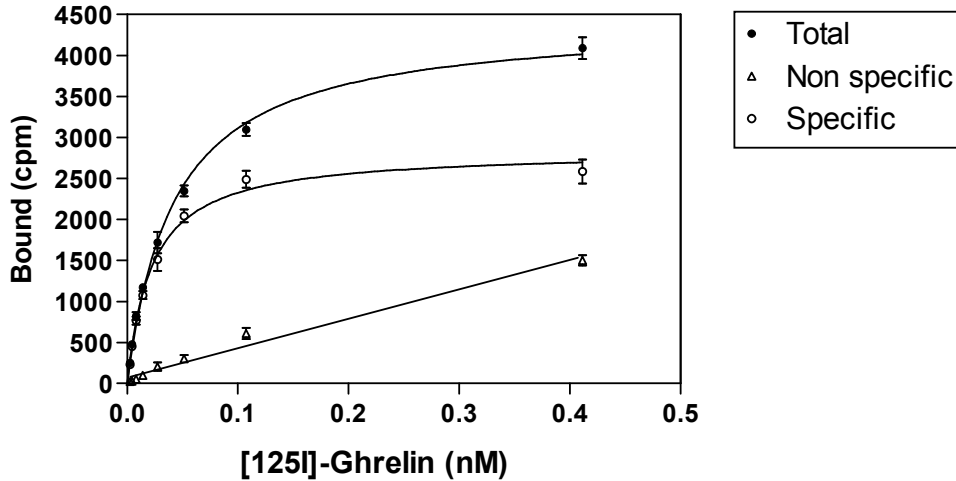
**ASSAY BUFFER:** 25 mM Hepes pH 7.4, 1 mM CaCl<sub>2</sub>, 5mM MgCl<sub>2</sub>, 2.5 mM EDTA, 0.4% BSA

**WASH BUFFER:** 50 mM Tris-HCl pH 7.4

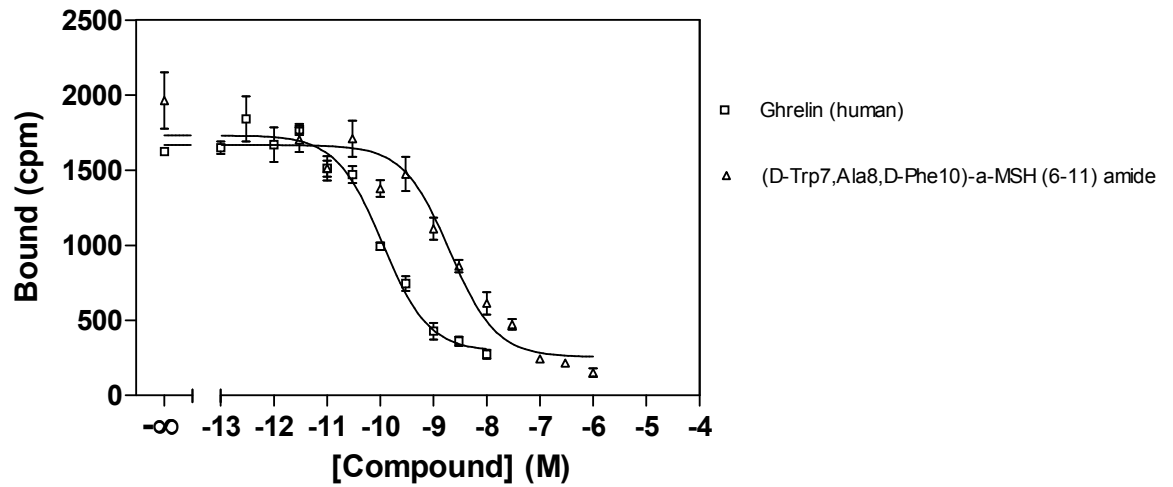
**BINDING PROTOCOL:** Binding assays are performed in 200 µl total volume according to the following conditions:

- 1 - Membrane dilution: 0.05 ml of membranes + 7.45 ml assay buffer (1:150 dilution)
- 2 - Incubation: 25 µl of incubation buffer or unlabeled ligand (Ghrelin (human), 1 µM final for non specific binding)  
25 µl of radioligand at the appropriate concentration (see graph on page 2)  
150 µl of diluted membranes
- 3 - Incubation time: 60 minutes at 27 °C
- 4 - Filtration: Over GF/C filter presoaked in 0.5 % PEI then washed 9x with 500 µl of ice cold wash buffer.

**LOT SPECIFIC DATA**



**Figure 1: SATURATION BINDING ASSAY CURVE (FILTRATION)**  
 96-well saturation binding assay curve (0.8 µg membranes/well, TopCount™).



**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 <sup>7</sup> ,Ala <sup>8</sup> ,D-Phe <sup>10</sup> )-α-MSH (6-11) amide	0.93

## SUGGESTED MATERIALS AND INSTRUMENTATION

	SUPPLIER	CATALOG #
<b>PHARMACOLOGY ASSAYS</b>		
• [ <sup>125</sup> I]-Ghrelin	PerkinElmer	NEX388
• Ghrelin (human)	Bachem	H-4864
• Unifilter-96 GF/C	PerkinElmer	6005174
• Unifilter-96 GF/B	PerkinElmer	6005177
• Filtermat A GF/C	PerkinElmer	1450-421
<b>INSTRUMENTS</b>		
• TopCount™	PerkinElmer	visit the web site or contact your local sales office
• Microbeta®	PerkinElmer	visit the web site or contact your local sales office
• FilterMate Unifiter 96-Harvester	PerkinElmer	C961962

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