

RBHGHS: Human Ghrelin Receptor (GHS-R)

SPECIFIC LOT DATA: Lot 394-585-A

Receptor Concentration (B_{max}): 1.4 pmol/mg protein

K_d for [¹²⁵I] Ghrelin binding: 0.0087 nM

Volume: 0.4 ml per 400 microassay size vial

Protein Concentration: 0.92 mg/ml

PACKAGING: Membranes suspended in 50 mM Tris-HCl pH 7.4, 10% sucrose

RECOMMENDED ASSAY PROCEDURE:

Thawing: Thaw vials rapidly; dilute with incubation buffer; homogenize. Keep on ice.

Incubation buffer: 25 mM Hepes, pH 7.4 @ 27°C, 5 mM MgCl₂, 1 mM CaCl₂, 2.5 mM EDTA and 0.4% BSA.

Binding protocol: Incubate 0.92 µg of membranes (20 µl of a 1:20 dilution in incubation buffer), buffer and [¹²⁵I] Ghrelin in a total volume of 300 µl.

Add the assay components in the following order:

- 200 µl incubation buffer;
- 40 µl 7.5 µM Ghrelin (1 µM final; for non-specific binding);
or 40 µl incubation buffer (for total binding);
- 40 µl [¹²⁵I] Ghrelin at the appropriate concentration (see graphs on reverse);
- 20 µl diluted membranes.

Incubate 60 min at 27°C.

Filter over GF/C filter (presoaked in 0.5% polyethylenimine) and wash filter 9x with 0.5 ml ice-cold 50 mM Tris-HCl, pH 7.4 @ 4°C.

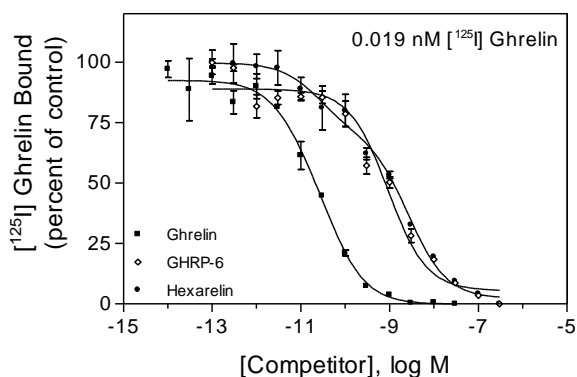
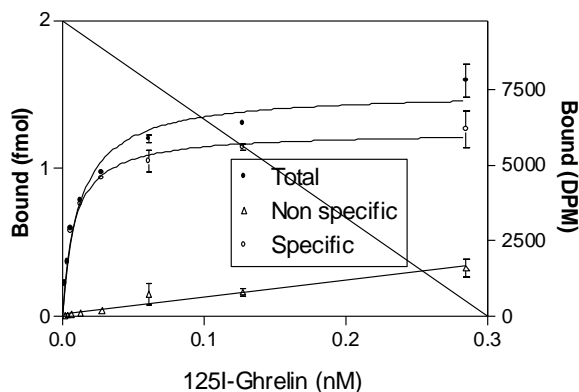
STABILITY AND STORAGE RECOMMENDATIONS: Storage at -80°C is recommended. Long term stability is currently under investigation. Repeated freeze-thawing of this product is not recommended.

ORIGIN: Human Ghrelin Receptor expressed in HEK-293 cells

REFERENCES: GenBank Accession #: U60179.
Howard et al, *Science*, 273:974-977 (1996).

[¹²⁵I] Ghrelin binding to human Ghrelin (GHS-R) Receptor

Lot No : 394-585-A



Affinity Constants for human
Ghrelin receptor
Lot 394-585-A

COMPETITOR*	Ki (nM)
Ghrelin	0.016
GHRP-6	0.58
Hexarelin	0.59

*Even though two sites can be observed occasionally with some ligands, the data presented is derived from single site fitting.

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