eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ Ghrelin Receptor Membrane Preparation

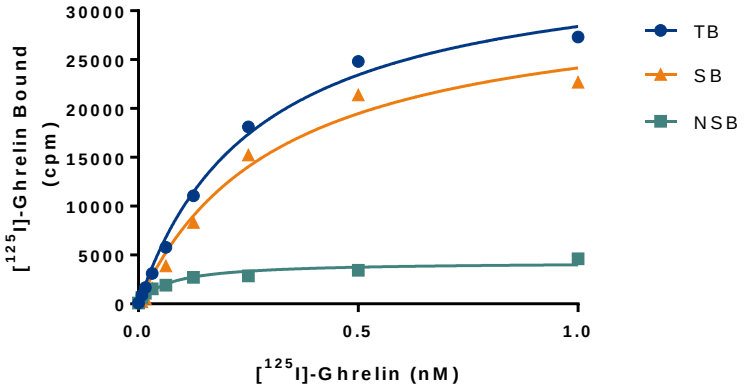
CATALOG NUMBER: HTS187M **QUANTITY:** 200 units

LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

BACKGROUND:

Ghrelin is a 28 amino acid peptide, containing a unique octanoyl moiety added post-translationally to Ser^3, with diverse activities in the CNS, gastrointestinal tract, and cardiovascular system (Davenport *et al*., 2005; Leite-Moreira and Soares, 2007). In the CNS, ghrelin stimulates release of growth hormone from the anterior pituitary and increases appetite by binding to neurons within the arcuate nucleus. Circulating concentrations of ghrelin increase with preprandially and decrease post-prandially, and thus counterbalances the effects of leptin to coordinate energy balance, appetite and food intake. Ghrelin is also expressed in the cardiovascular system where it acts as a potent vasodilator; receptors are up-regulated in patients with atherosclerosis, suggesting that it plays a role in compensating for increased vasoconstriction (Kleinz *et al*., 2006). The effects of ghrelin are mediated by a G_q-coupled receptor, originally designated GHSR (growth hormone secretogogue receptor), and more recently termed the Ghrelin Receptor or GRLN. Two splice variants have been described; type 1a (GHS-R1a) is the functional receptor, whereas type 1b (GHS-R1b) encodes a truncated, inactive protein with only 5 transmembrane domains. As administration of agonists of the ghrelin receptor to rats leads to increased food intake and antagonists reduce food intake (Beck *et al*. 2004), antagonism and inverse agonism of the ghrelin receptor may reduce food intake in certain types of obesity, and agonists of the ghrelin receptor are potentially useful for treatment of anorexia and cachexia. Ghrelin Receptor membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of antagonists of ghrelin interactions and its ligands. The membrane preparations exhibit a Kd of 0.32 nM for [^{125}I]-ghrelin. With 0.1 nM [^{125}I]-ghrelin, 5 µg/well Ghrelin Receptor Membrane Prep typically yields greater than 12-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay



Eurofins Pharma Bioanalytics Services US Inc.
6 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

Template version: 7. Modified Date: 26APR18. Modified by: JVK

Figure 1. Saturation binding for Ghrelin Receptor. 5.0 ug/well Ghrelin Receptor Membrane Preparation was incubated with increasing amount of [^{125}I]- ghrelin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled ghrelin. Specific binding (SB) was determined by subtracting NSB from TB.

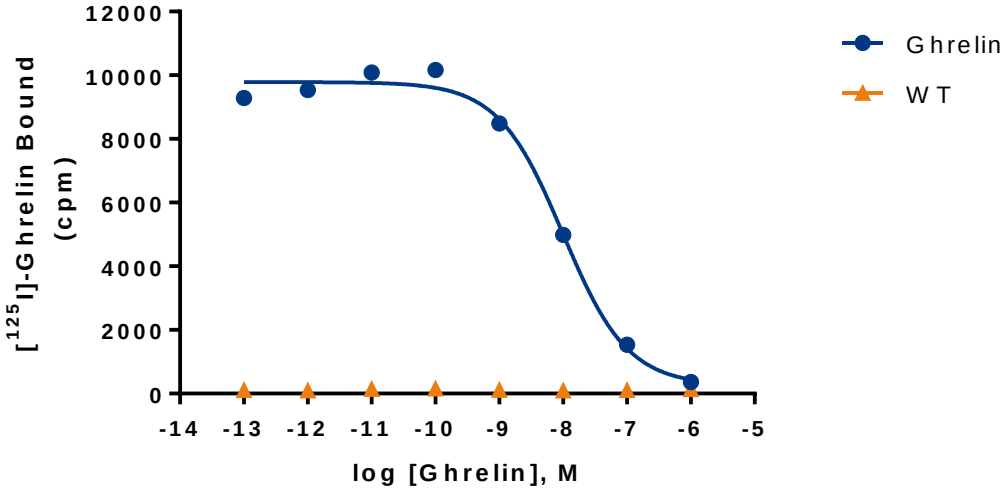


Figure 2. Competition binding for Ghrelin Receptor. 5µg/well Ghrelin Receptor Membrane Preparation and wild-type Chem-1 Membrane Preparation (Millipore catalog # HTS000MC1) were incubated in a 96-well plate with 0.1 nM ^{125}I-labeled ghrelin and increasing concentrations of unlabeled ghrelin. More than 12-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 4.2 pmol/mg protein
K_d: 0.32 nM
Signal:background: >12-fold

TRANSFECTION: Human GHSR cDNA encoding the ghrelin receptor isoform 1a(Accession Number: NM_198407)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous ghrelin receptor expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 50 mM Hepes, pH 7.4, 5 mM MgCl2, 1 mM CaCl2, 0.2% BSA, filtered and stored at 4°C

Radioligand: [125I]-Ghrelin. (Perkin Elmer#:NEX-338)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 12-fold signal:background with ^{125}I labeled ghrelin.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

Beck *et al.* (2004) Feeding response to ghrelin agonist and antagonist in lean and obese Zucker rats. *Life Sci.* 76(4): 473-8.

Davenport *et al.* (2005) International Union of Pharmacology. LVI. Ghrelin receptor nomenclature, distribution, and function. *Pharmacol. Rev.* 57(4): 541-6.

Kleinz *et al.* (2006) Functional and immunocytochemical evidence for a role of ghrelin and des-octanoyl ghrelin in the regulation of vascular tone in man. *Cardiovasc. Res.* 69(1): 227-35.

Leite-Moreira AF and Soares J-B (2007) Physiological, pathological and potential therapeutic roles of ghrelin. *Drug Discov. Today* 12: 276-288.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

Eurofins Pharma Bioanalytics Services US Inc. is an independent member of Eurofins Discovery Services