eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ Ghrelin Receptor Frozen Cells

CATALOG NUMBER: HTS187RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

Ghrelin is a 28 amino acid peptide, containing a unique octanoyl moiety added post-translationally to Ser3, 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 Gq-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. Cloned human ghrelin-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant ghrelin expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at Ghrelin Receptor.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

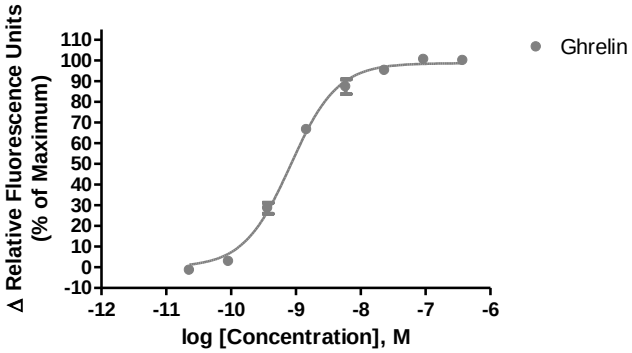

Figure 1. Representative data for activation of Ghrelin receptor. Calcium flux in Ghrelin–expressing Chem-1 cell line induced by Ghrelin. Ghrelin–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,500 RLU (Relative Light Units).

Table 1. Comparison of EC_{50} values of Ghrelin-expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Ghrelin	Calcium Flux	1	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Ghrelin ligand	Tocris: 1451
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for $FLIPR^{TETRA}$® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

GHSR cDNA (Accession Number: NM_198407; see CODING SEQUENCE below) expressed from a proprietary E5 promoter plasmid.

CODING SEQUENCE

```
ATG TGG AAC GCG ACG CCC AGC GAA GAG CCG GGG TTC AAC CTC ACA CTG GCC GAC CTG GAC TGG GAT GCT TCC
 M   W   N   A   T   P   S   E   E   P   G   F   N   L   T   L   A   D   L   D   W   D   A   S

CCC GGC AAC GAC TCG CTG GGC GAC GAG CTG CTG CAG CTC TTC CCC GCG CCG CTG CTG GCG GGC GTC ACA GCC
 P   G   N   D   S   L   G   D   E   L   L   Q   L   F   P   A   P   L   L   A   G   V   T   A

ACC TGC GTG GCA CTC TTC GTG GTG GGT ATC GCT GGC AAC CTG CTC ACC ATG CTG GTG GTG TCG CGC TTC CGC
 T   C   V   A   L   F   V   V   G   I   A   G   N   L   L   T   M   L   V   V   S   R   F   R

GAG CTG CGC ACC ACC ACC AAC CTC TAC CTG TCC AGC ATG GCC TTC TCC GAT CTG CTC ATC TTC CTC TGC ATG
 E   L   R   T   T   T   N   L   Y   L   S   S   M   A   F   S   D   L   L   I   F   L   C   M

CCC CTG GAC CTC GTT CGC CTC TGG CAG TAC CGG CCC TGG AAC TTC GGC GAC CTC CTC TGC AAA CTC TTC CAA
 P   L   D   L   V   R   L   W   Q   Y   R   P   W   N   F   G   D   L   L   C   K   L   F   Q

TTC GTC AGT GAG AGC TGC ACC TAC GCC ACG GTG CTC ACC ATC ACA GCG CTG AGC GTC GAG CGC TAC TTC GCC
 F   V   S   E   S   C   T   Y   A   T   V   L   T   I   T   A   L   S   V   E   R   Y   F   A

ATC TGC TTC CCA CTC CGG GCC AAG GTG GTG GTC ACC AAG GGG CGG GTG AAG CTG GTC ATC TTC GTC ATC TGG
 I   C   F   P   L   R   A   K   V   V   V   T   K   G   R   V   K   L   V   I   F   V   I   W

GCC GTG GCC TTC TGC AGC GCC GGG CCC ATC TTC GTG CTA GTC GGG GTG GAG CAC GAG AAC GGC ACC GAC CCT
 A   V   A   F   C   S   A   G   P   I   F   V   L   V   G   V   E   H   E   N   G   T   D   P

TGG GAC ACC AAC GAG TGC CGC CCC ACC GAG TTT GCG GTG CGC TCT GGA CTG CTC ACG GTC ATG GTG TGG GTG
 W   D   T   N   E   C   R   P   T   E   F   A   V   R   S   G   L   L   T   V   M   V   W   V

TCC AGC ATC TTC TTC TTC CTT CCT GTC TTC TGT CTC ACG GTC CTC TAC AGT CTC ATC GGC AGG AAG CTG TGG
 S   S   I   F   F   F   L   P   V   F   C   L   T   V   L   Y   S   L   I   G   R   K   L   W

CGG AGG AGG CGC GGC GAT GCT GTC GTG GGT GCC TCG CTC AGG GAC CAG AAC CAC AAG CAA ACC GTG AAA ATG
 R   R   R   R   G   D   A   V   V   G   A   S   L   R   D   Q   N   H   K   Q   T   V   K   M

CTG GCT GTA GTG GTG TTT GCC TTC ATC CTC TGC TGG CTC CCC TTC CAC GTA GGG CGA TAT TTA TTT TCC AAA
 L   A   V   V   V   F   A   F   I   L   C   W   L   P   F   H   V   G   R   Y   L   F   S   K

TCC TTT GAG CCT GGC TCC TTG GAG ATT GCT CAG ATC AGC CAG TAC TGC AAC CTC GTG TCC TTT GTC CTC TTC
 S   F   E   P   G   S   L   E   I   A   Q   I   S   Q   Y   C   N   L   V   S   F   V   L   F

TAC CTC AGT GCT GCC ATC AAC CCC ATT CTG TAC AAC ATC ATG TCC AAG AAG TAC CGG GTG GCA GTG TTC AGA
 Y   L   S   A   A   I   N   P   I   L   Y   N   I   M   S   K   K   Y   R   V   A   V   F   R

CTT CTG GGA TTC GAA CCC TTC TCC CAG AGA AAG CTC TCC ACT CTG AAA GAT GAA AGT TCT CGG GCC TGG ACA
 L   L   G   F   E   P   F   S   Q   R   K   L   S   T   L   K   D   E   S   S   R   A   W   T
GAA TCT AGT ATT AAT ACA TGA
 E   S   S   I   N   T  Stp
```

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS187M	ChemiScreen™ Ghrelin Receptor membrane prep

REFERENCES

1. Beck et al. (2004) Feeding response to ghrelin agonist and antagonist in lean and obese Zucker rats. *Life Sci*. 76(4): 473-8.
2. Davenport et al. (2005) International Union of Pharmacology. LVI. Ghrelin receptor nomenclature, distribution, and function. *Pharmacol. Rev.* 57(4): 541-6.
3. 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.
4. Leite-Moreira AF and Soares J-B (2007) Physiological, pathological and potential therapeutic roles of ghrelin. *Drug Discov. Today* 12: 276-288.
5. Matsumoto M et al. (2001) Structure-activity relationship of ghrelin: pharmacological study of ghrelin peptides. *Biochem. Biophys. Res. Commun.* 287: 142-146.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

User Agreement (Label License)

In addition to the General Terms and Conditions section, these specific terms also apply for **Ready-to-Assay™ Ghrelin Receptor Frozen Cells, Product No. HTS187RTA**

BY USING THE THIS PRODUCT LICENSED TO YOU ("LICENSEE") HEREUNDER, YOU ARE HEREBY REPRESENTING THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF OR YOUR COMPANY, AS APPLICABLE, AND ARE CONSENTING TO BE LEGALLY BOUND BY ALL OF THE TERMS OF THIS USER AGREEMENT ("AGREEMENT"). IF YOU DO NOT AGREE TO ALL THESE TERMS, DO NOT USE THE PRODUCT, AND IMMEDIATELY RETURN SUCH PRODUCTS TO THE APPLICABLE SELLER FOR A REFUND. This is a legal agreement between Licensee and Eurofins Pharma Bioanalytics Services US Inc. governing use of the Ready-to-Assay Cells products and/or any accompanying operating/use protocols (the "Product(s)") provided to Licensee.

LICENSEE shall obtain no ownership interest in the Product or use/culture protocols accompanying the Product other than through the perpetual limited license granted herein. If the Product is licensed through an authorized Eurofins Pharma Bioanalytics Services US Inc. distributor, Licensee shall be obligated to disclose its identity to Eurofins Pharma Bioanalytics Services US Inc. to insure compliance with this User Agreement.

Limited License and Restrictions. Pursuant to the terms and conditions of this Agreement, Eurofins Pharma Bioanalytics Services US Inc. conveys to Licensee the non-exclusive and non-transferable right to use the Licensed Product only for Research Purposes conducted by Licensee (whether Licensee is an academic user or a for-profit entity). "Research Purposes" means any biological research and development application or use, including without limitation, developing, demonstrating or validating biological assays, life sciences and/or pharmaceutical research. "Research Purposes" excludes applications outside biology (including but not limited to consumer electronics or materials sciences), and specifically excludes the following applications of whatever kind or nature: Clinical Diagnostics (any use of a product or service for clinical diagnosis where data from an individual's sample is given to such individual or used for the purpose of diagnosis or treatment of a medical condition in such individual, where that result may be used in the treatment of such individual), therapeutics, clinical imaging, environmental testing and cosmetics. Contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee. Licensee cannot sell or otherwise transfer (a) this Product or (b) materials made using this Product to a third party. Licensee may transfer information or materials made through use of this Product to a scientific collaborator, provided that such transfer is not for the commercial purposes, and that such collaborator agrees in writing: (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for Research Purposes and not for commercial purposes. Commercial purposes means any activity by a user of the

Product for consideration that may include, but is not limited to: (1) operating a service business that uses the Products to develop information or data which is resold for research and development applications; (2) use of the Product in manufacturing; (3) use of the Product for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the Product, whether or not such Product is resold for use in research. Licensee expressly represents and warrants to Eurofins Pharma Bioanalytics Services US Inc. that Licensee will properly test and use any Product purchased from Eurofins Pharma Bioanalytics Services US Inc. or its affiliated companies in accordance with the practices of a reasonable person who is an expert in the field and in strict compliance with all applicable laws and regulations, now and hereinafter enacted. Licensee agrees to comply with instructions, if any, furnished by Eurofins Pharma Bioanalytics Services US Inc. relating to the use of the Product and to not misuse the Product in any manner. Licensee shall not reverse engineer, disassemble or modify the Product or create any derivative works of the written materials accompanying the Product, including but not limited to any material data sheets or similar materials with respect to the Products' specifications. Licensee acknowledges that Eurofins Pharma Bioanalytics Services US Inc. or its affiliated companies retains ownership of all patents, copyrights, trademarks, trade secrets and other proprietary rights relating to or residing in the Product or any portion thereof.

Term and Termination. This Agreement commences upon Licensee's use of the Products, and shall remain in effect in perpetuity unless terminated sooner as set forth hereunder. Eurofins Pharma Bioanalytics Services US Inc. may terminate this Agreement immediately if Licensee breaches any provision herein. Upon any such termination, all rights granted to Licensee hereunder will immediately terminate, and Licensee shall immediately cease using the Product and, at Eurofins Pharma Bioanalytics Services US Inc.'s option, return or destroy all Products (certifying such destruction to Eurofins Pharma Bioanalytics Services US Inc. in writing).

Assignment. Licensee shall not sublicense, assign (by operation of law of otherwise) or otherwise transfer this Agreement or any of the rights or licenses granted under this Agreement without the prior written consent of Eurofins Pharma Bioanalytics Services US Inc.. Any attempted assignment, sublicense or transfer by Licensee without such consent shall be null and void.