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PRODUCT DATASHEET

ChemiScreen™ Ghrelin Receptor Stable Cell Line

CATALOG NUMBER: HTS187C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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. The cloned human Ghrelin Receptor-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant Ghrelin Receptor expression on the cell surface and contains high levels of the promiscuous G protein to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at Ghrelin Receptor.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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.

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APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

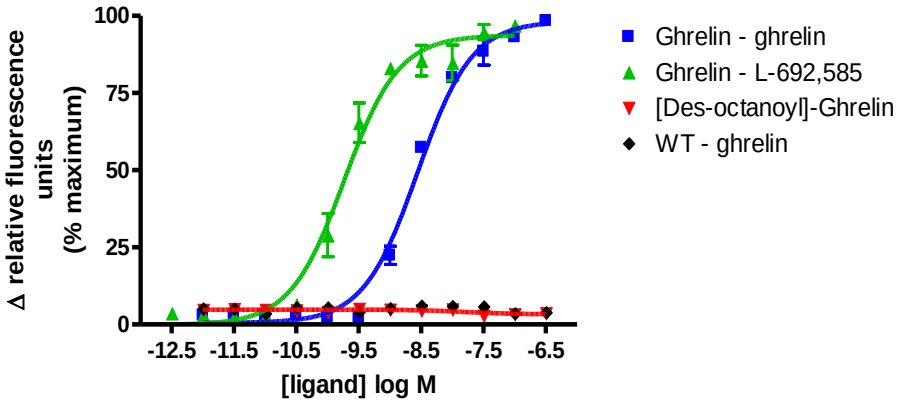

Figure 1. Representative data for activation of the Ghrelin receptor stably expressed in Chem-1 cells induced by Ghrelin using a fluorescent calcium flux assay. Ghrelin–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of Ghrelin-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Ghrelin	Calcium Flux - Fluorescence	2.79	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence. The Z' value, as defined with response to 10µM 2MeSATP, was 0.71.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Ghrelin ligand	Tocris: 1463
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

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HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human Ghrelin cDNA (Accession Number: NM_198407; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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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
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (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. Leite-Moreira AF and Soares J-B (2007) Physiological, pathological and potential therapeutic roles of ghrelin. *Drug Discov. Today* 12: 276-288.

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