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PRODUCT DATASHEET

ChemiScreen™ GIP Glucagon Receptor Stable Cell Line

CATALOG NUMBER: HTS134C

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-9 host, which supports high levels of functional receptor expression on the cell surface. Chem-9 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

Gastric inhibitory polypeptide receptor (GIP) has been identified in the glucose-mediated secretion of insulin (Mayo *et al.*, 2003). GIP is in the secretin/VIP receptor family which includes secretin, VIP, glucagon, GLP-1, growth hormone releasing hormone (GHRH), and PACAP (Yip *et al.*, 1999). GIP is secreted after meal ingestion has been shown to stimulate bone formation resulting in lower occurrences of osteoporosis (Tsukiyama *et al.*, 2006). Type 2 diabetes is a result of decreased glucose-stimulated insulin secretion which makes insulin secretion potentiators a popular target for diabetes treatments, it is thought that a defect in GIP expression and/or signaling may lead to cell dysfunction and type 2 diabetes (Mayo *et al.*, 2003). The cloned human GIP–expressing cell line is made in the Chem-9 host, which supports high levels of recombinant GIP expression on the cell surface and contains high levels of promiscuous G proteins to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between GIP and its ligands.

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, cAMP accumulation

APPLICATION DATA

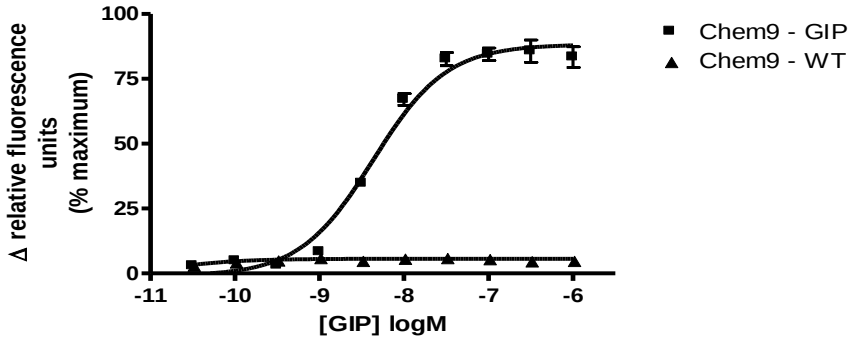

Figure 1. Representative data for activation of the GIP receptor stably expressed in Chem-9 cells induced by GIP using a fluorescent calcium flux assay. GIP–expressing Chem-9 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 7,000 RLU. Similarly parental cells (catalog #: HTSCHEM-9) were tested to determine the specificity of the resulting signal.

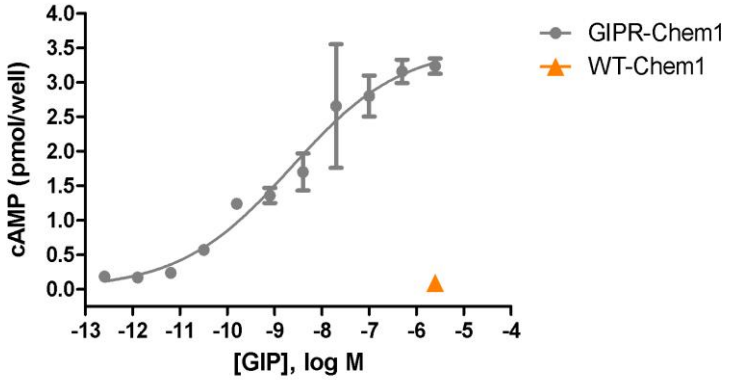

Figure 2. Representative data for activation of GIP receptor stably expressed in Chem-9 cells induced by GIP ligand using a cAMP accumulation assay. GIP–expressing Chem-9 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were treated with GIP ligand for 10 minutes in the presence of 100 µM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation or [with GIP Ligand for 10 minutes in the presence of 100 µM IBMX and 10 µM forskolin, to stimulate adenylate cyclase, and 0.5% DMSO to determine the receptor's ability to inhibit cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal fluorescence signal obtained in this experiment was 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-9) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of GIP-expressing Chem-9 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
GIP	Calcium Flux - Fluorescence	4	Eurofins Internal Data
GIP	cAMP accumulation	2	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.

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
	Hygromycin	250 µg/ml	Invivogen: ant-hg-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	0.65	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
GIP ligand	Tocris: 2084
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.

HOST CELL

Chem-9, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human GIP cDNA (Accession Number: NM_000164; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

```
                                        ATG ACT ACC TCT CCG ATC CTG CAG CTG CTG CTG CGG CTC TCA CTG
                                         M   T   T   S   P   I   L   Q   L   L   L   R   L   S   L

TGC GGA CTG CTG CTC CAG AGG GCG GAG ACA GGC TCT AAG GGG CAG ACG GCG GGG GAG CTG TAC CAG CGC
 C   G   L   L   L   Q   R   A   E   T   G   S   K   G   Q   T   A   G   E   L   Y   Q   R

TGG GAA CGG TAC CGC AGG GAG TGC CAG GAG ACC TTG GCA GCC GCG GAA CCG CCT TCA GGC CTC GCC TGT
 W   E   R   Y   R   R   E   C   Q   E   T   L   A   A   A   E   P   P   S   G   L   A   C

AAC GGG TCC TTC GAT ATG TAC GTC TGC TGG GAC TAT GCT GCA CCC AAT GCC ACT GCC CGT GCG TCC TGC
 N   G   S   F   D   M   Y   V   C   W   D   Y   A   A   P   N   A   T   A   R   A   S   C

CCC TGG TAC CTG CCC TGG CAC CAC CAT GTG GCT GCA GGT TTC GTC CTC CGC CAG TGT GGC AGT GAT GGC
 P   W   Y   L   P   W   H   H   H   V   A   A   G   F   V   L   R   Q   C   G   S   D   G

CAA TGG GGA CTT TGG AGA GAC CAT ACA CAA TGT GAG AAC CCA GAG AAG AAT GAG GCC TTT CTG GAC CAA
 Q   W   G   L   W   R   D   H   T   Q   C   E   N   P   E   K   N   E   A   F   L   D   Q

AGG CTC ATC TTG GAG CGG TTG CAG GTC ATG TAC ACT GTC GGC TAC TCC CTG TCT CTC GCC ACA CTG CTG
 R   L   I   L   E   R   L   Q   V   M   Y   T   V   G   Y   S   L   S   L   A   T   L   L

CTA GCC CTG CTC ATC TTG AGT TTG TTC AGG CGG CTA CAT TGC ACT AGA AAC TAT ATC CAC ATC AAC CTG
 L   A   L   L   I   L   S   L   F   R   R   L   H   C   T   R   N   Y   I   H   I   N   L

TTC ACG TCT TTC ATG CTG CGA GCT GCG GCC ATT CTC AGC CGA GAC CGT CTG CTA CCT CGA CCT GGC CCC
 F   T   S   F   M   L   R   A   A   A   I   L   S   R   D   R   L   L   P   R   P   G   P

TAC CTT GGG GAC CAG GCC CTT GCG CTG TGG AAC CAG GCC CTC GCT GCC TGC CGC ACG GCC CAG ATC GTG
 Y   L   G   D   Q   A   L   A   L   W   N   Q   A   L   A   A   C   R   T   A   Q   I   V

ACC CAG TAC TGC GTG GGT GCC AAC TAC ACG TGG CTG CTG GTG GAG GGC GTC TAC CTG CAC AGT CTC CTG
 T   Q   Y   C   V   G   A   N   Y   T   W   L   L   V   E   G   V   Y   L   H   S   L   L

GTG CTC GTG GGA GGC TCC GAG GAG GGC CAC TTC CGC TAC TAC CTG CTC CTC GGC TGG GGG GCC CCC GCG
 V   L   V   G   G   S   E   E   G   H   F   R   Y   Y   L   L   L   G   W   G   A   P   A

CTT TTC GTC ATT CCC TGG GTG ATC GTC AGG TAC CTG TAC GAG AAC ACG CAG TGC TGG GAG CGC AAC GAA
 L   F   V   I   P   W   V   I   V   R   Y   L   Y   E   N   T   Q   C   W   E   R   N   E

GTC AAG GCC ATT TGG TGG ATT ATA CGG ACC CCC ATC CTC ATG ACC ATC TTG ATT AAT TTC CTC ATT TTT
 V   K   A   I   W   W   I   I   R   T   P   I   L   M   T   I   L   I   N   F   L   I   F

ATC CGC ATT CTT GGC ATT CTC CTG TCC AAG CTG AGG ACA CGG CAA ATG CGC TGC CGG GAT TAC CGG CTG
 I   R   I   L   G   I   L   L   S   K   L   R   T   R   Q   M   R   C   R   D   Y   R   L

AGG CTG GCT CGC TCC ACG CTG ACG CTG GTG CCC CTG CTG GGT GTC CAC GAG GTG GTG TTT GCT CCC GTG
 R   L   A   R   S   T   L   T   L   V   P   L   L   G   V   H   E   V   V   F   A   P   V

ACA GAG GAA CAG GCC CGG GGC GCC CTG CGC TTC GCC AAG CTC GGC TTT GAG ATC TTC CTC AGC TCC TTC
 T   E   E   Q   A   R   G   A   L   R   F   A   K   L   G   F   E   I   F   L   S   S   F
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CAG GGC TTC CTG GTC AGC GTC CTC TAC TGC TTC ATC AAC AAG GAG GTG CAG TCG GAG ATC CGC CGT GGC
 Q   G   F   L   V   S   V   L   Y   C   F   I   N   K   E   V   Q   S   E   I   R   R   G

TGG CAC CAC TGC CGC CTG CGC CGC AGC CTG GGC GAG GAG CAA CGC CAG CTC CCG GAG CGC GCC TTC CGG
 W   H   H   C   R   L   R   R   S   L   G   E   E   Q   R   Q   L   P   E   R   A   F   R

GCC CTG CCC TCC GGC TCC GGC CCG GGC GAG GTC CCC ACC AGC CGC GGC TTG TCC TCG GGG ACC CTC CCA
 A   L   P   S   G   S   G   P   G   E   V   P   T   S   R   G   L   S   S   G   T   L   P

GGG CCT GGG AAT GAG GCC AGC CGG GAG TTG GAA AGT TAC TGC TGA
 G   P   G   N   E   A   S   R   E   L   E   S   Y   C  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-9	ChemiScreen™ Chem-9 Parental Cell Line (control cells)
HTS134M	ChemiScreen™ GIP Glucagon Receptor Membrane Prep

REFERENCES

1. Mayo KE *et al.* (2003). International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol. Rev.* 55: 167-194.
2. Tsukiyama K *et al.* (2006). Gastric Inhibitory Polypeptide as an Endogenous Factor Promoting New Bone Formation after Food Ingestion. *Mol. Endocrin.* 20(7): 1644-1651.
3. Yip RGC *et al.* (1999). GIP Biology and Fat Metabolism. *Life Sci.* 66(2): 91-103.

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