eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ GIP Glucagon Receptor Frozen Cells

CATALOG NUMBER: HTS134RTA

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.

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 β-cell dysfunction and type 2 diabetes (Mayo *et al.*, 2003). 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 optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at GIP.

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.

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APPLICATIONS

Calcium Flux Fluorescent Assays, cAMP Accumulation Assays

APPLICATION DATA

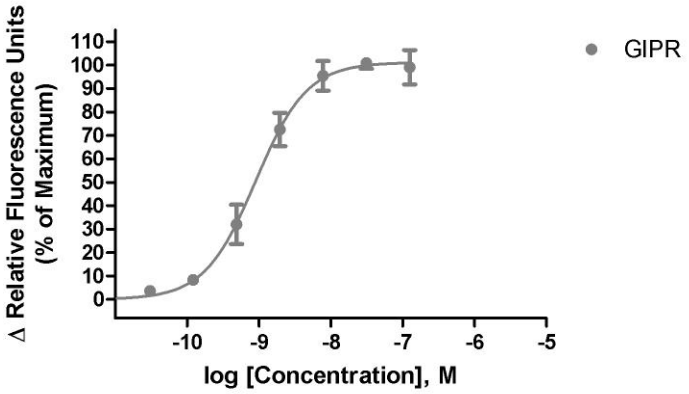

Figure 1. Representative data for activation of GIP receptor. Calcium flux in GIP–expressing Chem-9 cell line induced by GIP. GIP–expressing Chem-9 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,000 RLU (Relative Light Units).

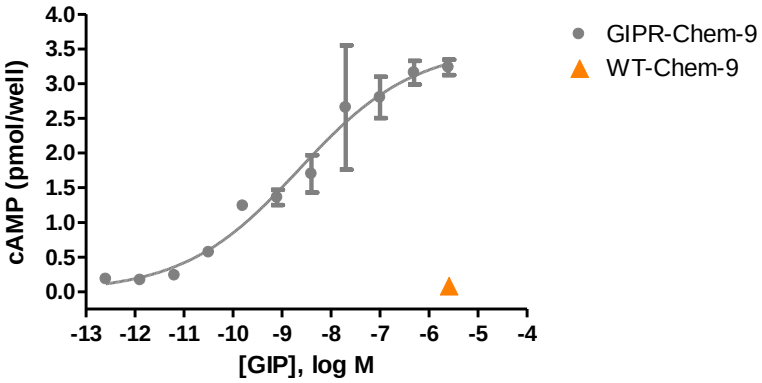

Figure 1. Representative data for activation of GIP receptor stably expressed in Chem-9 cells induced by GIP using a cAMP accumulation assay. GIPR–expressing Chem-9 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with GIP for 15 minutes in the presence of 2.0 mM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal cAMP response obtained in this experiment was 3.5 pmol/well. Similarly parental cells were tested to determine the specificity of the resulting signal.

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
GIP	Calcium Flux	1	Eurofins Internal Data
GIP	cAMP accumulation	2	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
U-50488 ligand	Tocris: 2084
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-9, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein as well as an exogenous proprietary promiscuous Gα protein.

EXONGENOUS GENE EXPRESSION

GIPR cDNA (Accession Number: NM_000164; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                        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
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 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

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-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (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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