eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ GLP_2 Glucagon Receptor Stable Cell Line

CATALOG NUMBER: HTS164C

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-11 host, which supports high levels of functional receptor expression on the cell surface. Chem-11 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

Glucagon-like peptide 2 (GLP-2) is a 33 amino acid peptide secreted by intestinal endocrine L cells in response to nutrient uptake. GLP-2 exerts multiple effects on intestinal physiology, including promotion of epithelial cell growth and survival to increase intestinal mass, increasing nutrient absorption capacity, and inhibition of intestinal inflammation (Dubé and Brubaker, 2007). The GLP-2 Receptor is a class B GPCR belonging to the glucagon/secretin family of receptors, and as with the other members of the family, activation of the GLP-2 receptor results in increased intracellular cAMP (Munroe *et al*., 1999). The cloned human GLP-2 Receptor -expressing cell line is made in the Chem-11 host, which supports high levels of recombinant GLP-2 Receptor expression on the cell surface and contains optimized levels of a proprietary promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at GLP-2 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, cAMP accumulation

APPLICATION DATA

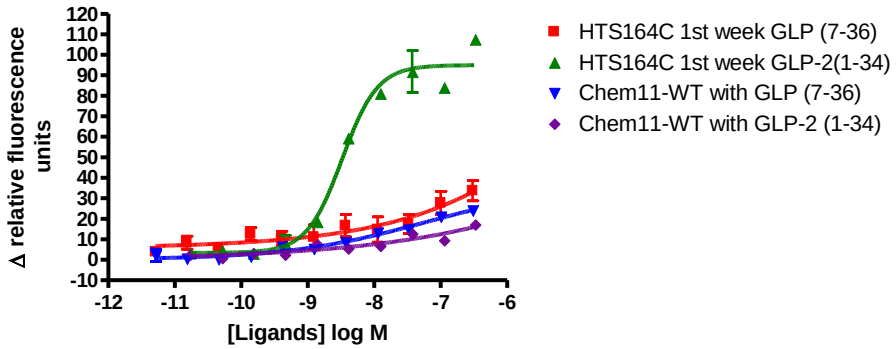

Figure 1. Representative data for activation of the GLP_2 receptor stably expressed in Chem-11 cells induced by GLP_2 Ligand using a fluorescent calcium flux assay. GLP_2–expressing Chem-11 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with 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-11) were tested to determine the specificity of the resulting signal.

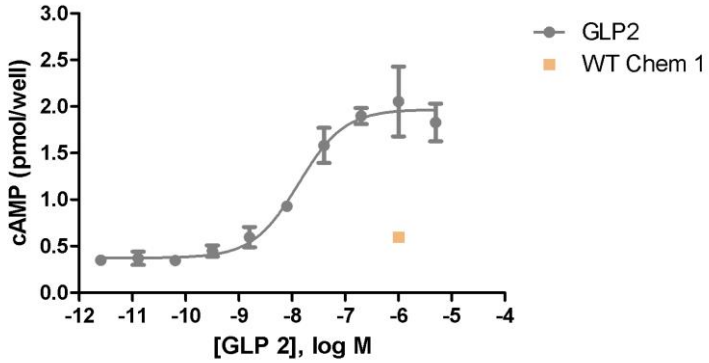

Figure 2. Representative data for activation of GLP_2 receptor stably expressed in Chem-11 cells induced by GLP_2 Ligand using a cAMP accumulation assay. GLP_2–expressing Chem-11 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were treated with Ligand for 10 minutes in the presence of 100 µM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation or with 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 7,000 RLU. Similarly parental cells (catalog #: HTSCHEM-11) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of GLP_2-expressing Chem-11 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
GLP_2	Calcium Flux - Fluorescence	3	Eurofins Internal Data
GLP_2	cAMP accumulation	13	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 0.58.

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
GLP_2 ligand	Tocris: 2258
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. Invert plate and blot on absorbent pad to remove medium from assay plate.
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-11, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human GLP_2 cDNA (Accession Number: NM_004246; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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ATG AAG CTG GGA TCG AGC AGG GCA GGG CCT GGG AGA GGA AGC GCG GGA CTC CTG CCT GGC GTC CAC GAG     69
 M   K   L   G   S   S   R   A   G   P   G   R   G   S   A   G   L   L   P   G   V   H   E     23

CTG CCC ATG GGC ATC CCT GCC CCC TGG GGG ACC AGT CCT CTC TCC TTC CAC AGG AAG TGC TCT CTC TGG    138
 L   P   M   G   I   P   A   P   W   G   T   S   P   L   S   F   H   R   K   C   S   L   W     46

GCC CCT GGG AGG CCC TTC CTC ACT CTG GTC CTG CTG GTT TCC ATC AAG CAA GTT ACA GGA TCC CTC CTT    207
 A   P   G   R   P   F   L   T   L   V   L   L   V   S   I   K   Q   V   T   G   S   L   L     69

GAG GAA ACG ACT CGG AAG TGG GCT CAG TAC AAA CAG GCA TGT CTG AGA GAC TTA CTC AAG GAA CCT TCT    276
 E   E   T   T   R   K   W   A   Q   Y   K   Q   A   C   L   R   D   L   L   K   E   P   S     92

GGC ATA TTT TGT AAC GGG ACA TTT GAT CAG TAC GTG TGT TGG CCT CAT TCT TCT CCT GGA AAT GTC TCT    345
 G   I   F   C   N   G   T   F   D   Q   Y   V   C   W   P   H   S   S   P   G   N   V   S    115

GTA CCC TGC CCT TCA TAC TTA CCT TGG TGG AGT GAA GAG AGC TCA GGA AGG GCC TAC AGA CAC TGC TTG    414
 V   P   C   P   S   Y   L   P   W   W   S   E   E   S   S   G   R   A   Y   R   H   C   L    138

GCT CAG GGG ACT TGG CAG ACG ATA GAG AAC GCC ACG GAT ATT TGG CAG GAT GAC TCC GAA TGC TCC GAG    483
 A   Q   G   T   W   Q   T   I   E   N   A   T   D   I   W   Q   D   D   S   E   C   S   E    161

AAC CAC AGC TTC AAG CAA AAC GTG GAT CGT TAT GCC TTG CTG TCA ACC TTG CAG CTG ATG TAC ACC GTG    552
 N   H   S   F   K   Q   N   V   D   R   Y   A   L   L   S   T   L   Q   L   M   Y   T   V    184

GGA TAC TCC TTC TCT CTT ATC TCC CTC TTC CTG GCT CTC ACC CTC CTC TTG TTT CTT CGA AAA CTC CAC    621
 G   Y   S   F   S   L   I   S   L   F   L   A   L   T   L   L   L   F   L   R   K   L   H    207

TGC ACG CGC AAC TAC ATC CAC ATG AAC TTG TTT GCT TCT TTC ATC CTG AGA ACC CTG GCT GTA CTG GTG    690
 C   T   R   N   Y   I   H   M   N   L   F   A   S   F   I   L   R   T   L   A   V   L   V    230

AAG GAC GTC GTC TTC TAC AAC TCT TAC TCC AAG AGG CCT GAC AAT GAG AAT GGG TGG ATG TCC TAC CTG    759
 K   D   V   V   F   Y   N   S   Y   S   K   R   P   D   N   E   N   G   W   M   S   Y   L    253

TCA GAG ATG TCC ACC TCC TGC CGC TCA GTC CAG GTT CTC TTG CAT TAC TTT GTG GGT GCC AAT TAC TTA    828
 S   E   M   S   T   S   C   R   S   V   Q   V   L   L   H   Y   F   V   G   A   N   Y   L    276

TGG CTG CTG GTT GAA GGC CTC TAC CTC CAC ACG CTG CTG GAG CCC ACA GTG CTT CCT GAG AGG CGG CTG    897
 W   L   L   V   E   G   L   Y   L   H   T   L   L   E   P   T   V   L   P   E   R   R   L    299

TGG CCC AGA TAC CTG CTG TTG GGT TGG GCC TTC CCT GTG CTA TTT GTT GTA CCC TGG GGT TTC GCC CGT    966
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 W   P   R   Y   L   L   L   G   W   A   F   P   V   L   F   V   V   P   W   G   F   A   R     322
GCA CAC CTG GAG AAC ACA GGG TGC TGG ACA ACA AAT GGG AAT AAG AAA ATC TGG TGG ATC ATC CGA GGA    1035
 A   H   L   E   N   T   G   C   W   T   T   N   G   N   K   K   I   W   W   I   I   R   G     345

CCC ATG ATG CTC TGT GTA ACA GTC AAT TTC TTC ATC TTC CTG AAA ATT CTC AAG CTT CTC ATT TCT AAG    1104
 P   M   M   L   C   V   T   V   N   F   F   I   F   L   K   I   L   K   L   L   I   S   K     368

CTC AAA GCT CAT CAA ATG TGC TTC AGA GAT TAT AAA TAC AGA TTG GCA AAA TCA ACA CTG GTC CTC ATT    1173
 L   K   A   H   Q   M   C   F   R   D   Y   K   Y   R   L   A   K   S   T   L   V   L   I     391

CCT TTA TTG GGC GTT CAT GAG ATC CTC TTC TCT TTC ATC ACT GAT GAT CAA GTT GAA GGA TTT GCA AAA    1242
 P   L   L   G   V   H   E   I   L   F   S   F   I   T   D   D   Q   V   E   G   F   A   K     414

CTT ATA CGA CTT TTC ATT CAG TTG ACA CTG AGC TCC TTT CAT GGG TTC CTG GTG GCC TTG CAG TAT GGT    1311
 L   I   R   L   F   I   Q   L   T   L   S   S   F   H   G   F   L   V   A   L   Q   Y   G     437

TTT GCC AAT GGA GAG GTG AAG GCT GAG CTG CGG AAA TAC TGG GTC CGC TTC TTG CTA GCC CGC CAC TCA    1380
 F   A   N   G   E   V   K   A   E   L   R   K   Y   W   V   R   F   L   L   A   R   H   S     460

GGC TGC AGA GCC TGT GTC CTG GGG AAG GAC TTC CGG TTC CTA GGA AAA TGT CCC AAG AAG CTC TCG GAA    1449
 G   C   R   A   C   V   L   G   K   D   F   R   F   L   G   K   C   P   K   K   L   S   E     483

GGA GAT GGC GCT GAG AAG CTT CGG AAG CTG CAG CCC TCA CTT AAC AGT GGG CGG CTC CTA CAT CTA GCC    1518
 G   D   G   A   E   K   L   R   K   L   Q   P   S   L   N   S   G   R   L   L   H   L   A     506

ATG CGA GGT CTT GGG GAG CTG GGC GCC CAG CCC CAA CAG GAC CAT GCA CGC TGG CCC CGG GGC AGC AGC    1587
 M   R   G   L   G   E   L   G   A   Q   P   Q   Q   D   H   A   R   W   P   R   G   S   S     529

CTG TCC GAG TGC AGT GAG GGG GAT GTC ACC ATG GCC AAC ACC ATG GAG GAG ATT CTG GAA GAG AGT GAG    1656
 L   S   E   C   S   E   G   D   V   T   M   A   N   T   M   E   E   I   L   E   E   S   E     552

ATC TGA
 I  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-11	ChemiScreen™ Chem-11 Parental Cell Line (control cells)
HTS164M	ChemiScreen™ GLP_2 Glucagon Receptor Membrane Prep

REFERENCES

1. Dubé PE and Brubaker PL (2007) Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. *Am. J. Physiol. Endocrinol. Metab.* 293: E460-E465.
2. Munroe DG *et al*. (1999) Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. *Proc. Natl. Acad. Sci. USA* 96: 1569-1573.

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