eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ GLP-2 Glucagon Receptor Frozen Cells

CATALOG NUMBER: HTS164RTA

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 over night recovery, assays for calcium response.

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). Millipore's cloned human GLP-2 -expressing cell line is made in the Chem-11 host, which supports high levels of recombinant GLP-2 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 GLP-2.

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.

APPLICATIONS

Calcium Flux Assays, cAMP accumulation

APPLICATION DATA

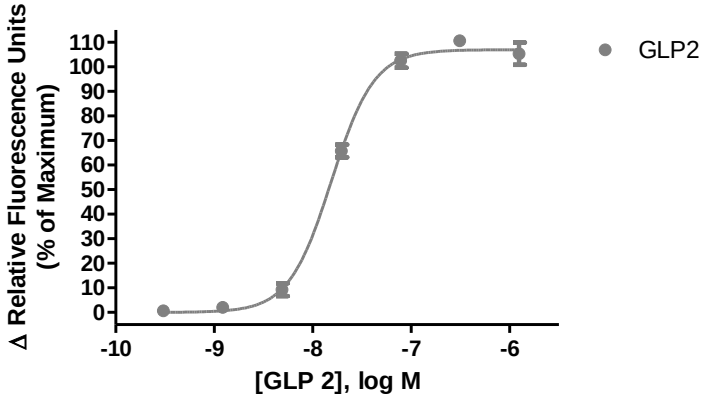

Figure 1. Representative data for activation of GLP-2 receptor. Calcium flux in GLP-2–expressing Chem-11 cell line induced by GLP-2. GLP-2–expressing Chem-11 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 35,000 RLU (Relative Light Units).

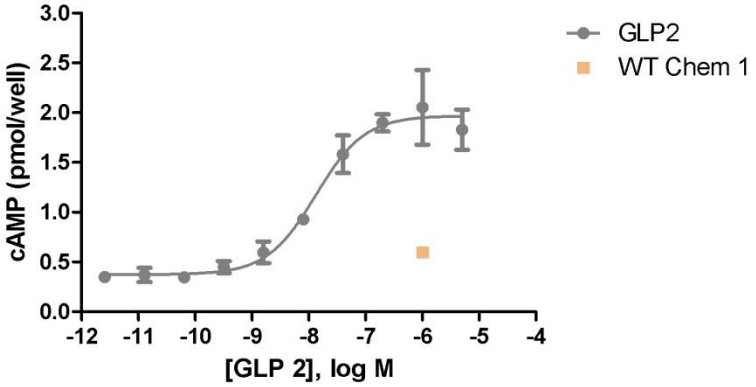

Figure 2. Representative data for activation of GLP2 receptor stably expressed in CHEM-11 cells induced by GLP 2 using a cAMP accumulation assay. GLP2–expressing CHEM-11 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with GLP2 for 15 minutes 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 2.5 pmol/well. Similarly parental cells (catalog #: HTSCHEM -1) were tested to determine the specificity of the resulting signal.

Table I. Comparison of EC_{50} values of GLP-2-expressing Chem-11 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
GLP-2	Calcium Flux	15	Eurofins Internal Data
GLP-2	cAMP accumulation	13	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: SH3026802
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
U-50488 ligand	Sigma: D8040
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/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-11, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein as well as an exogenous proprietary promiscuous Gα protein.

EXONGENOUS GENE EXPRESSION

GLP2R cDNA (Accession Number: NM_004246; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

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
 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
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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
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GGC TGC AGA GCC TGT GTC CTG GGG AAG GAC TTC CGG TTC CTA GGA AAA TGT CCC AAG AAG CTC TCG GAA   1449
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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-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

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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