

PRODUCT DATASHEET

Ready-to-Assay™ Glucagon Receptor Frozen Cells

CATALOG NUMBER: HTS112RTA

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 N₂. Media Component at 4°C (-20°C 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.

Glucagon is a 29-amino acid peptide that stimulates glycogenolysis and gluconeogenesis in the liver to increase blood glucose. The receptor for glucagon is a class 2 (or class B) GPCR that signals through G_s to stimulate cAMP production (Mayo et al., 2003). Mice lacking the glucagon receptor have mild hypoglycemia after fasting, and exhibit hyperplasia of pancreatic α -cells (Gelling et al., 2003). Because of its role in promoting hyperglycemia, the glucagon receptor presents a potential target for treatment of diabetes. Cloned human Glucagon-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant Glucagon expression on the cell surface and contains high levels of the promiscuous G protein G α 15 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 Glucagon.

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

APPLICATION DATA

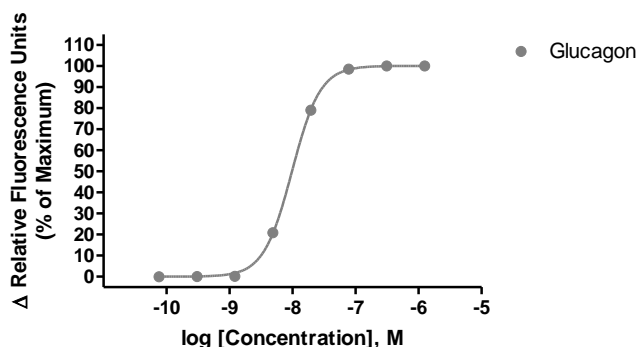


Figure 1. Representative data for activation of Glucagon receptor. Calcium flux in Glucagon-expressing Chem-1 cell line induced by Glucagon. Glucagon-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4200 RLU (Relative Light Units).

Table 1. Summary of EC₅₀ value of Glucagon-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Glucagon	Calcium Flux	10	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 37°C 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 37°C 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) Ca²⁺ dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca²⁺ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca²⁺ 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 Ca²⁺ 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
Probenecid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Glucagon ligand	Sigma: G2044
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-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

GCGR cDNA (Accession Number: NM_000160; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG CCC CCC TGC CAG CCA CAG CGA CCC CTG CTG CTG
M P P C Q P Q R P L L L

TTG CTG CTG CTG CTG GCC TGC CAG CCA CAG GTC CCC TCC GCT CAG GTG ATG GAC TTC CTG TTT GAG AAG
L L L L L A C Q P Q V P S A Q V M D F L F E K

TGG AAG CTC TAC GGT GAC CAG TGT CAC CAC AAC CTG AGC CTG CTG CCC CCT CCC ACG GAG CTG GTG TGC
W K L Y G D Q C H H N L S L L P P P T E L V C

AAC AGA ACC TTC GAC AAG TAT TCC TGC TGG CCG GAC ACC CCC GCC AAT ACC ACG GCC AAC ATC TCC TGC
N R T F D K Y S C W P D T P A N T T A N I S C

CCC TGG TAC CTG CCT TGG CAC CAC AAA GTG CAA CAC CGC TTC GTG TTC AAG AGA TGC GGG CCC GAC GGT
P W Y L P W H H K V Q H R F V F K R C G P D G

CAG TGG GTG CGT GGA CCC CGG GGG CAG CCT TGG CGT GAT GCC TCC CAG TGC CAG ATG GAT GGC GAG GAG
Q W V R G P R G Q P W R D A S Q C Q M D G E E

ATT GAG GTC CAG AAG GAG GTG GCC AAG ATG TAC AGC AGC TTC CAG GTG ATG TAC ACA GTG GGC TAC AGC
I E V Q K E V A K M Y S S F Q V M Y T V G Y S

CTG TCC CTG GGG GCC CTG CTC CTC GCC TTG GCC ATC CTG GGG GGC CTC AGC AAG CTG CAC TGC ACC CGC
L S L G A L L L A L A I L G G L S K L H C T R

AAT GCC ATC CAC GCG AAT CTG TTT GCG TCC TTC GTG CTG AAA GCC AGC TCC GTG CTG GTC ATT GAT GGG
N A I H A N L F A S F V L K A S S V L V I D G

CTG CTC AGG ACC CGC TAC AGC CAG AAA ATT GGC GAC GAC CTC AGT GTC AGC ACC TGG CTC AGT GAT GGA
L L R T R Y S Q K I G D D L S V S T W L S D G

GCG GTG GGT GGC TGC CGT GTG GCC GCG GTG TTC ATG CAA TAT GGC ATC GTG GCC AAC TAC TGC TGG CTG
A V A G C R V A A V F M Q Y G I V A N Y C W L

CTG GTG GAG GGC CTG TAC CTG CAC AAC CTG CTG GGC CTG GCC ACC CTC CCC GAG AGG AGC TTC TTC AGC
L V E G L Y L H N L L G L A T L P E R S F F S

CTC TAC CTG GGC ATC GGC TGG GGT GCC CCC ATG CTG TTC GTC GTC CCC TGG GCA GTG GTC AAG TGT CTG
L Y L G I G W G A P M L F V V P W A V V K C L

TTC GAG AAC GTC CAG TGC TGG ACC AGC AAT GAC AAC ATG GGC TTC TGG TGG ATC CTG CGG TTC CCC GTC
F E N V Q C W T S N D N M G F W W I L R F P V

TTC CTG GCC ATC CTG ATC AAC TTC TTC ATC TTC GTC CGC ATC GTT CAG CTG CTC GTG GCC AAG CTG CGG
F L A I L I N F F I F V R I V Q L L V A K L R

GCA CGG CAG ATG CAC CAC ACA GAC TAC AAG TTC CGG CTG GCC AAG TCC ACG CTG ACC CTC ATC CCT CTG
A R Q M H H T D Y K F R L A K S T L T L I P L

T-C
CTG GGC GTC CAC GAA GTG GTC TTC GCC TTC GTG ACG GAC GAG CAC GCC CAG GGC ACC CTG CGC TCC GCC
L G V H E V V F-F A F V T D E H A Q G T L R S A

AAG CTC TTC TTC GAC CTC TTC CTC AGC TCC TTC CAG GGC CTG CTG GTG GCT GTC CTC TAC TGC TTC CTC
K L F F D L F L S S F Q G L L V A V L Y C F L

AAC AAG GAG GTG CAG TCG GAG CTG CGG CGG CGT TGG CAC CGC TGG CGC CTG GGC AAA GTG CTA TGG GAG
N K E V Q S E L R R R W H R W R L G K V L W E

GAG CGG AAC ACC AGC AAC CAC AGG GCC TCA TCT TCG CCC GGC CAC GGC CCT CCC AGC AAG GAG CTG CAG
E R N T S N H R A S S S P G H G P P S K E L Q

TTT GGG AGG GGT GGT GGC AGC CAG GAT TCA TCT GCG GAG ACC CCC TTG GCT GGT GGC CTC CCT AGA TTG
F G R G G G S Q D S S A E T P L A G G L P R L

GCT GAG AGC CCC TTC TGA
A E S P F Stp

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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS112C	ChemiScreen™ GCG Glucagon receptor stable cell line
HTS112M	ChemiScreen™ GCG Glucagon receptor membrane prep
HTS112LT	ChemiBrite™ GCG Glucagon receptor frozen cells

REFERENCES

1. Gelling RW et al. (2003) Lower blood glucose, hyperglucagonemia, and pancreatic α cell hyperplasia in glucagon receptor knockout mice. *Proc. Natl. Aca. Sci. USA* 100:1438-1443.
2. Mayo KE et al. (2003) International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol. Rev.* 55: 167-194.

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