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Glucagon-Like Peptide 2 Receptor (GLP2R) ACTOne™ Stable Cell Line

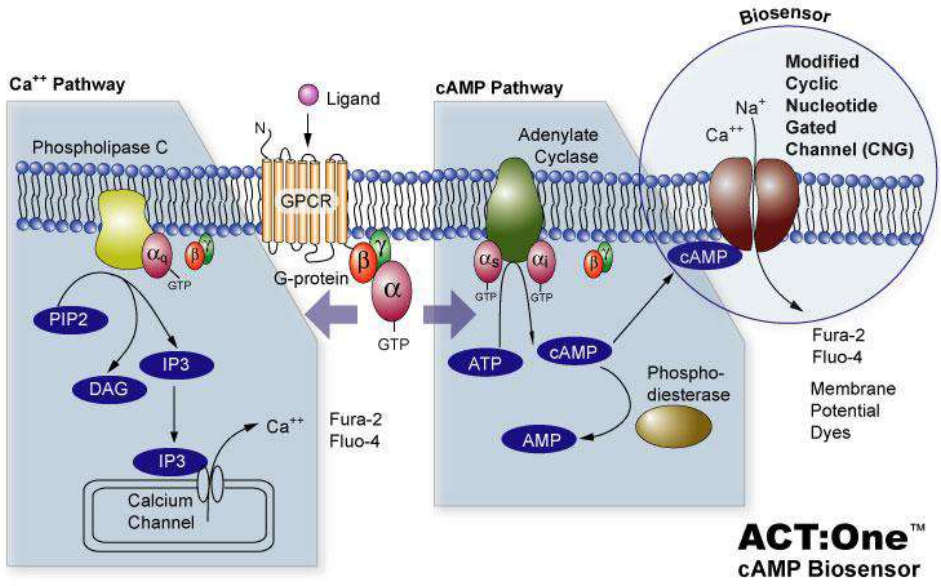
CATALOG NUMBER: CL-01-GLP2R

Introduction

GLP2R is a G protein-coupled receptor superfamily member closely related to the glucagon receptor (GLP1 receptor). Glucagon-like peptide-2 (GLP2) is a 33-amino acid proglucagon-derived peptide produced by intestinal enteroendocrine cells. Like glucagon-like peptide-1 (GLP1) and glucagon itself, it is derived from the proglucagon peptide encoded by the GCG gene. GLP2 stimulates intestinal growth and up-regulates villus height in the small intestine, concomitant with increased crypt cell proliferation and decreased enterocyte apoptosis. Moreover, GLP2 prevents intestinal hypoplasia resulting from total parenteral nutrition. GLP2R, a G protein-coupled receptor superfamily member is expressed in the gut and closely related to the glucagon receptor (GCGR) and the receptor for GLP1 (GLP1R)**.**

Description

Human GLP2R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human GLP2R. HEK-293 CNG cells express a modified CNG (Cyclic Nucleotide Gated) channel that opens in response to elevated intracellular cAMP levels and consequently result in ion flux (often detectable by calcium-responsive dye, Cat# CA-C155) and cell membrane depolarization which can be easily measured with fluorescent Membrane Potential Dye (Cat# CA-M165). The assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FLIPR, or a fluorescence microplate reader.



Parental Cells

HEK-293 CNG cells (originally developed by BD Biosciences by introducing CNG in HEK-293 cells) (Cat# CL-03-PC20)

Gene/Enzyme Introduced

GLP2R (Genebank Accession No. NP_004237.1)

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Applications

- cAMP dependent human GLP2R cell based assay
- cell based high-throughput screening of human GLP2R inhibitors

Functional Test

- this cell line has been tested positive for GLP2R specific response
- surviving rate: More than 2.5 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of mycoplasma contamination.

Content

- Stable cells: 1 mL (1 x 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)
- Parental cells: 1 mL (1 x 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium (**for Stable Cell Line**): DMEM-10% FBS supplemented with 250 µg/ml G418, 1 µg/ml Puromycin
- Growth medium (**for Parental Cell Line**): DMEM-10% FBS supplemented with 250 µg/ml G418
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Assay materials not included：

10X Elite™ Membrane Potential Assay Kit	EENZYME Cat# CA-M165
Biocoat Poly-D-Lysine coated 384-well black/clear plate	BD 354663
Phosphodiesterase (PDE) inhibitor Ro 20-1724 (50mM stock in DMSO, store at -20°C)	Sigma B8279
Dulbecco's Phosphate Buffered Saline (DPBS)	Sigma D8537
GLP-2 (100µM stock in dH_2O)	American Peptide 46-0-80

Cell culture materials not included：

DMEM, high glucose, with glutamine	Biosource International P104G-000
Fetal bovine serum	Invitrogen 26140-079
Trypsin-EDTA solution (10x)	Sigma T4174
G418 sulfate	Cellgro 61-234-RG
Puromycin	Clontech 8052-2

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Cell Culture Protocol

Note. *Please finish reading the whole protocol before beginning the experiment.*

THAWING AND PLATING CELLS (REQUIRES 1-3 DAYS)

1. Prepare complete cell culture medium consisting of 90% Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), 250 μg/ml G418 and 1 □g/ml puromycin. Warm the medium to ~37°C.

2. Remove a vial of cells from the liquid Nitrogen tank. Wear safety glasses and always point the cap away from your face when opening.

3. Place the vial of cells in a 37ºC water bath until just thawed (less than 5 minutes). Immediately transfer cells to a 10 cm cell-culture plate or a T25 flask with 9 ml of the appropriate culture medium (pre-warmed to 37°C).

4. Place the cells in a cell-culture incubator at 37°C with 5% CO_2 for 4 hrs.

5. After 4 hours replace the culture medium with appropriate fresh culture medium (pre-warmed at 37°C).

6. Place the cells back in the incubator for 1-3 days. The cells will not require feeding before they reach 80-90% confluence and are ready for expansion. Split the cells when they reach 80-90% confluence.

Note. *It is very important that the cells DO NOT reach >90% confluence. Over-confluent growth can result in a significantly reduced response to ligands, and it may take several passages for the cells to recover to optimal stage.*

SPLITTING AND AMPLIFYING CELLS (REQUIRES 1-3 DAYS)

1. Remove the culture medium and replace it with a volume of Dulbecco's Phosphate Buffers Saline without calcium and magnesium (DPBS) to adequately cover and wash the cells. Remove DPBS.

2. Add a sufficient volume of 1x trypsin-EDTA to just cover the cells (i.e. 1 ml for a 10 cm dish, 2 ml for a T25 flask, and 5 ml for a T75 flask) Rock the plate to make sure the cells are equally covered with the solution. Trypsinize the cells at room temperature for ~ 5 min. After 5 min, check the cells to ensure that they are coming off the dish/flask. Tap the dish/flask gently to aid in the process. Add enough serum-containing medium to give a volume of ~ 10 ml, and pipette the medium up and down through a 10 ml serological pipette ~ 4 times to obtain a single cell suspension. Remove a portion of the sample for a cell count.

Note. *Trypsin is required to dissociate the cells during the process of passage. Cells may not be able to recover to an optimal stage if trypsin- free dissociation buffer is used.*

3. For primary expansion from a frozen vial, reseed the total cell volume in a T150 flask. For routine cell passage, split the cells using a ratio of 1:4 – 1:10.

4. Observe the cells daily and harvest the cells when they reach 80-90% confluence (1-3 days). Cells do not need to be fed during this time. Do not allow the cells to grow over 90% confluence.

FREEZING AND STORING THE CELLS

1. Remove cells from T75 flask by trypsinization as described above. Add 10 ml culture medium, and break the cell clumps via pipetting. Count cells using a hemocytometer.

2. Place cell suspension in a sterile centrifuge tube, and pellet the cells at ~ 200X g at 4°C for 5 min. Remove the medium, and resuspend the cell pellet in an appropriate volume of freezing medium (90% FBS and 10% DMSO) to give a cell density of 2.5 X 10^6 cells/ml.

3. Dispense the cells in 1 ml aliquots into cryo storage vials to give 2.5 X 10^6 cells/vial.

4. Freeze the cells in a cryo freezing-container overnight at -80ºC.

5. Next day, transfer the cell vials to a liquid nitrogen tank for long-term storage.

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ACTOne cAMP Assay Protocol

Note: *Please finish reading the whole protocol before you start the experiment !!!*

CELL PREPARATION

1) Harvest cells when they reach 80-90% confluence in flasks. Trypsinize cells as described in Cell Culture Protocol above. Count a portion of the cells with a hemocytometer.

   Note. *It is very important that the cells DO NOT reach >90% confluence. Over-confluent growth can result in a significantly reduced response to ligands, and it may take several passages for the cells to recover to optimal stage.*

2) The cell number needs to be optimized for each assay. Optimal assay conditions require a confluent monolayer of cells prior to the assay. Poly-D-Lysine coated plates are recommended to improve cell attachment. Plate 70K cells/well for a 96 well plate and 14K cells/well for a 384 well plate the day before the experiment. Add 100 µl/well of cell suspension to 96-well plates or 20 µl/well to 384-well plates.

3) Allow cells to attach by leaving the cell plates at room temperature for 30 minutes. Transfer the plates to a cell culture incubator and grow the cells overnight.

PREPARATION of 1X DYE-LOADING SOLUTION

1) Remove a bottle of 10X Elite™ Membrane Potential Dye Solution from -20˚C, and allow it to thaw at room temperature.

2) To prepare 1X Elite™ Membrane Potential Dye Dilution Buffer, transfer 10 ml of 10X Elite™ Membrane Potential Dye Dilution Buffer to 90 ml of deionized-distilled or reverse osmosis H_2O. Mix well.

3) To prepare 1X Dye-loading Solution for 10 cell plates, pipette 10 ml of 10X Elite™ Membrane Potential Dye Solution to 90 ml of 1X Elite™ Membrane Potential Dye Dilution Buffer. Mix well.

4) Aliquot the unused 1X Dye-loading Solution to several tubes, and store at -20°C. Store 1X Elite™ Membrane Potential Dye Dilution Buffer at room temperature.

   Note: *1x Dye-loading Solution is stable for more than one month if stored at -20°C. Avoid repetitive freeze-thaw cycles.*

DYE LOADING

1) Add phosphodiesterase inhibitor Ro20-1724 to a concentration of 50 µM in 1X Dye-loading Solution (optional step).

   Note: *Adding a phosphodiesterase inhibitor will increase the assay sensitivity.*

2) Remove cell plates from incubator and add an equal volume of 1 X Dye-loading Solution to each well (e.g. 100 µl to 100 µl culture medium/well for 96-well plates, or 20 µl to 20 µl culture medium/well for 384-well plates).

   Note: *For some assays that require a serum-free environment, culture medium that contains serum should be removed prior to dye loading and replaced with a equal volume of 1X DPBS buffer containing 0.2 to 0.5% BSA.*

3) Incubate cell plates with dye for 2 hour at room temperature in the dark.

PREPARATION of COMPOUND PLATES

Dilute 100 µM GLP-2 stock in DPBS with 2.5% DMSO as shown in Table 1. These concentrations are 5X the expected final testing concentrations.

Note: *DPBS with 2.5%DMSO helps increase solubility of GLP-2.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	50	15	5	1.5	0.5	0.15	0.05	0.015	0.005	0.0015	0.0005	0

---------- GLP-2 (nM)----------

Table 1. *An example of GLP-2 concentrations in a compound dilution plate*

If an antagonist assay is performed, the concentrations listed below must be adjusted to accommodate the volume of antagonist added.

Dilute antagonists in DPBS to a desired concentration. Add to cell plates loaded with dye.
Incubate the plates at room temperature for approximately 15 min prior to addition of the specific agonists.

Note: *Antagonist incubation times should be optimized for each assay.*

MEMBRANE POTENTIAL ASSAY

Kinetic assay with on-line compound addition

- Place the cell plates on a FLIPR, FlexStation or FDSS, and perform the membrane potential assay with on-line addition of test compounds in 1X DPBS to the cell plates at 50 μl/well for 96-well plates (250 μl total well volume after addition) or 10 μl/well for 384-well plates (50 μl total well volume after addition).

The final concentrations of GLP-2 used are listed in Table 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	3	1	0.3	0.1	0.03	0.01	0.003	0.001	0.0003	0.0001	0

---------- GLP-2 (nM)----------

Table 2. An example of the final testing concentrations of GLP-2 in a cell assay plate

- For assays performed on a **FLIPR**, use the 540-590 bandpass FLIPR filter (#2) from Molecular Devices (cat. #0310-4077).
- For assays performed on a **FlexStation**, use the following wavelength parameters: Excitation: 530 nm; Emission: 565 nm; Auto Cutoff: on (550 nm)

  Note: *Dispense speed and height for compound additions need to be optimized for each instrument.*

Endpoint assay or kinetic assay with off-line compound addition

- A fluorescence plate reader with bottom read-head is required. Test filter settings to optimize fluorescence light collection and eliminate bleedthrough of excitation light to emission filters. For example, the following filter settings can be used with excitation = 530/20 nm and emission = 590/20 nm.
- Read fluorescent baseline and record the fluorescent counts prior to drug addition. Add test compounds to cell plates off-line, and then read the plates at the appropriate time after drug addition.

  Note: *Dispense speed and height for compound additions need to be optimized so that the cell monolayer is not disturbed.*

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APPENDIX

Product Information

Catalog Number:	**CL-01-GLP2R**
Components:	**GLP2R cell line, 2 vials, frozen**

CELL LINE DESIGNATION	Glucagon-like peptide 2 receptor cell line
ORIGIN (PARENTAL CELL)	HEK 293-CNG cell (Cat# CL-03-PC20)
GENE INTRODUCED	Genbank LocusID 9340
RECEPTOR INTRODUCED:	Human glucagon-like peptide 2 receptor (NCBI protein database NP_004237.1)

USAGE

- cAMP assay for Gs-coupled human glucagon-like peptide 2 receptor (GLP2R)
- HEK293-CNG cells (Cat# CL-03-PC20) without transfected Glucagon-like peptide 2 receptor are used as a negative control.

QUALITY CONTROL

1. This cell line has been tested negative for *Mycoplasma sp*.
2. This cell line has been tested positive for glucagon-like peptide 2 receptor specific response.
3. Surviving rate: More than 2.5 million/vial on the second day after thawing.
4. The receptor specific activity is stable for 10 weeks continuous passage.

CELL CULTURE CONDITION

1. Growth medium: 90% DMEM, 10% FBS, 250 μg/ml G418 and 1 μg/ml puromycin
2. Freezing medium: 10% DMSO, 90% FBS

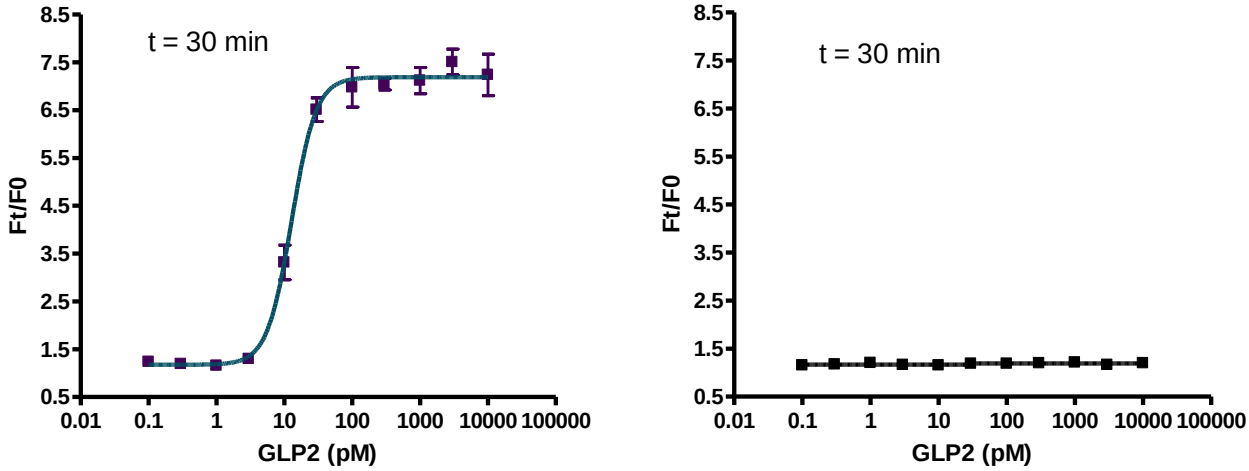

Figure 1. Response of ACT*One*™ GLP2R cell line & parental cell line to GLP-2.

ACTOne™ GLP2R cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hour of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of GLP-2. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of GLP-2 in ACT*One*™ GLP2R cell line. EC50 = 12.8 pM in the absence of 25 µM Ro20-1724 (data shown).

B. Parental cells do not respond to GLP-2.

TROUBLESHOOTING GUIDE

1. **Low survival rate of cells after thawing**
   - Cell vials could have thawed accidentally. Store cell vials in liquid nitrogen immediately after receiving and keep frozen at all times.
   - Leaving the vial at 37°C for too long during thawing will lower the survival rate. Place the vial at 37°C until cells are just thawed.
   - Handle the cells gently. Don't tap the vial or pipette the cells too many times before plating the cells.
   - Replace the medium four hours after thawing or when the cells have settled to remove DMSO.

2. **Slow growth rate of cells**
   - Do not split cells before they have completely recovered from thawing and reach at least 50% confluence**.**
   - Do not dilute cells excessively while splitting.
   - Spit cells before they reach 80 – 90% confluence.
   - Use Trypsin-EDTA solution to dissociate cells.
   - Cells may not be able to recover to an optimal stage if trypsin- free dissociation buffer is used.

3. **High baseline fluorescent signal**
   - Inspect the cell density and morphology under a microscope. High cell density or unhealthy cells can result in high baseline signal.
   - Do not remove serum-containing medium from cell plates before dye-loading. If a serum-free environment is required, use DPBS buffer containing 0.2 to 0.5% BSA to replace medium.

4. **Response to agonist is lower than expected**
   - Check the overall health of cells.
   - Cell density is too high or too low. Cell number titration may be necessary.
   - Keep cells growing in medium with proper drug selection.
   - Check settings of fluorescence readers.

5. **High well-to-well variations.**
   - Cells should be evenly distributed among wells. Before plating, microscopically examine the culture to be sure that they have been dissociated into single cells. Leave the cell plates at room temperature for 30 minutes prior to transferring the plates to a cell culture incubator.
   - Check the liquid handling system for dispensing accuracy. Optimize the settings of liquid handling system so that cell monolayer is not disturbed by dye and compound addition.
   - Check settings of fluorescence readers.

6. **Response from cells after the addition of buffer containing only DMSO**
   - Keep the final DMSO concentration below 1%.

Notice to Purchaser