

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

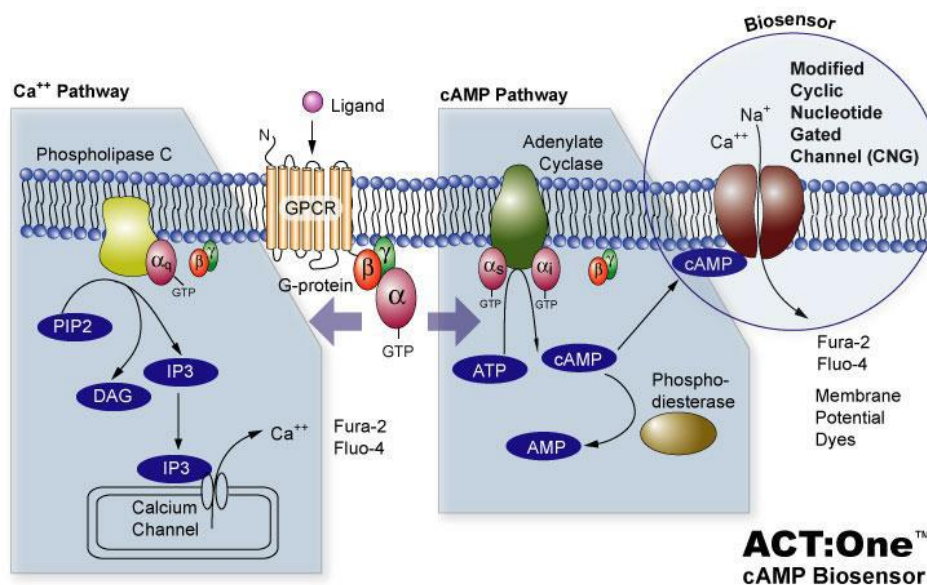
CATALOG NUMBER: CL-01-GLP2R

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 receptor. 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 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 FDSS, 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 (Genbank Accession No. NP_004237.1)

Applications

- cAMP dependent human GLP2R receptor cell based assay
- cell based high-throughput screening of human GLP2R receptor agonists/antagonists

Functional Tests

- this cell line has been tested positive for GLP2R receptor specific response
- surviving rate: more than 2 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 have been tested and found to be free of mycoplasma contamination.

Content

- Stable GLP2R receptor cells: 1 mL (2 x 10⁶ cells/mL in DMEM, 10% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 µg/ml G418, 1 µg/ml Puromycin
- 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 dH2O) | 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 |



DATA EXAMPLE

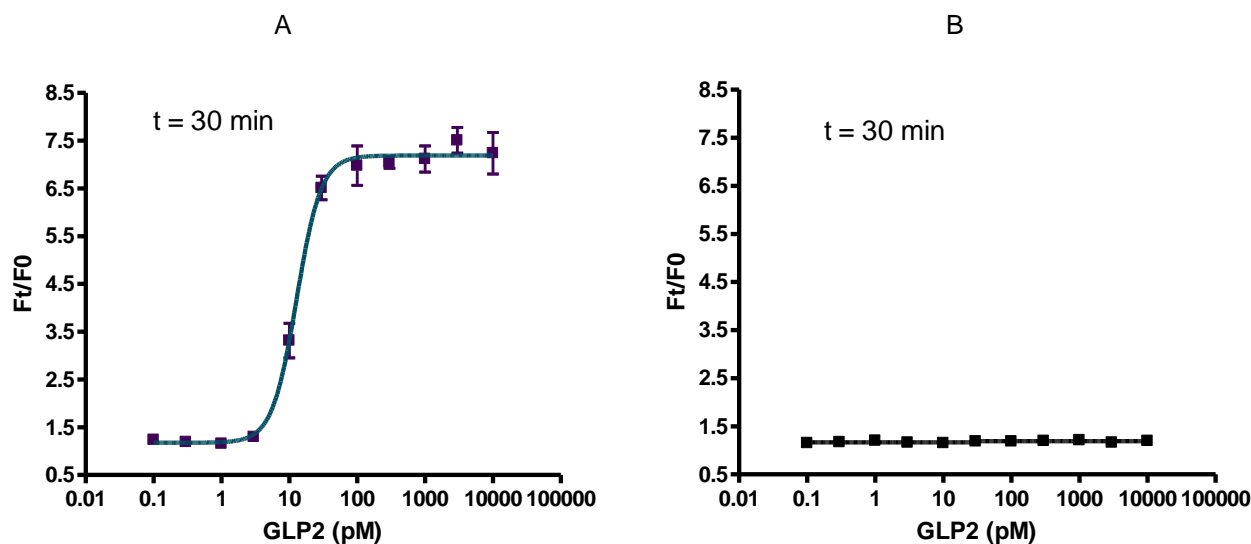


Figure 1. Response of ACTOne™ GLP2R cell line & parental cell line to GLP-2.

ACTOne™ GLP2R cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 μ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 μ l/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hours 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 ACTOne™ GLP2R cell line. EC₅₀ = 12.8 pM in the presence of 25 μ M PDE inhibitor Ro20-1724.
- B. Parental cells do not respond to GLP-2.

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