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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

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- Mindermengenzuschlag
- Trockeneiszuschlag
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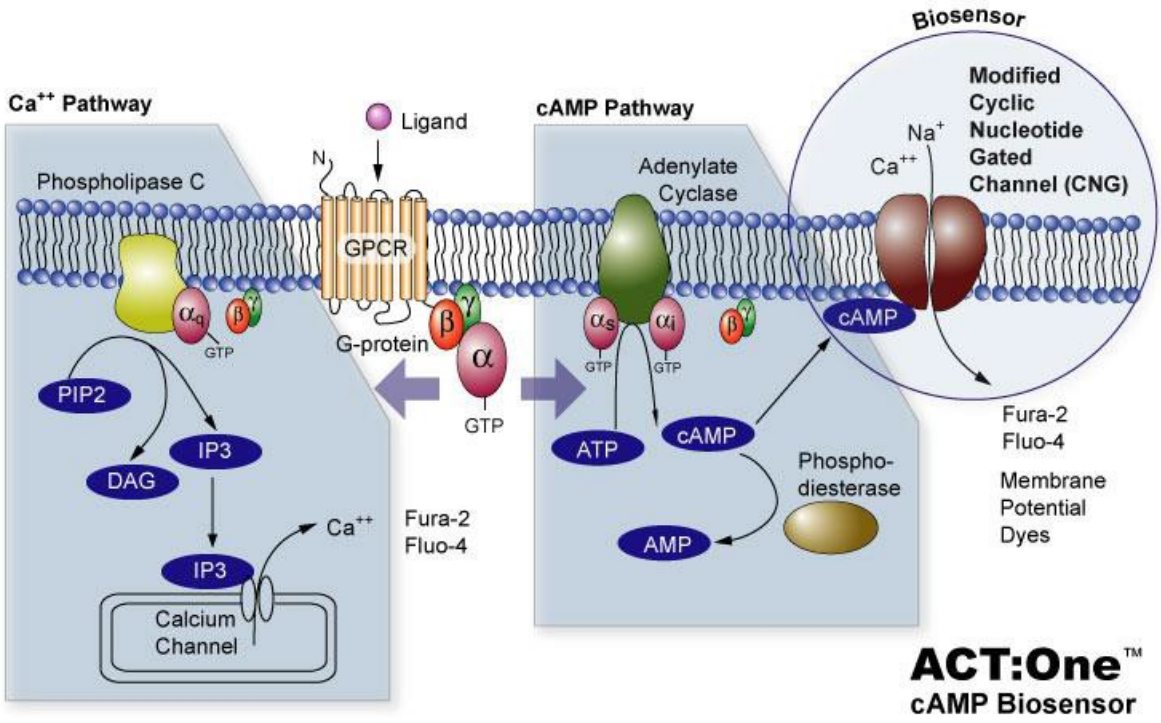
Human Luteinizing Hormone/Choriogonadotropin Receptor (LHCGR) ACTOne™ Stable Cell Line

CATALOG NUMBER: CL-01-LHCGR

Description

Human LHCGR ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human LHCGR. 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 FDSS, FLIPR, or a fluorescence microplate reader. It is a simple homogenous assay involving only dye and compound addition steps, allowing easy implementation in a high-throughput environment.



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

LHCGR

Tel: 1-800-919-0755 • Fax: 1-240-683-5852 • info@eEnzyme.com • www.eEnzyme.com

Applications

- cAMP dependent human luteinizing hormone/choriogonadotropin receptor cell based assay
- cell based high-throughput screening of human LHCGR receptor agonists/antagonists

Mycoplasma Contamination Test

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

Content

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

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: 90%DMEM, 10% FBS, supplemented with 250 µg/ml G418 and 1 µg/ml Puromycin.
- Freezing medium: 10% DMSO, 90% FBS

Subculturing Procedure

1. Thaw the frozen cryovial of cells within 1-2 min by gentle agitation in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% ethanol and transfer into a 75 cm^2 flask with 20 ml of complete DMEM growth medium.
2. Remove and discard culture medium next day, and then add fresh DMEM complete medium.
3. Monitor cell density daily. Cells should be passaged (1:3) when the culture reaches 90% confluence. Expected cell yield is between 1.5 x 10^5 and 2x 10^5 viable cells/cm^2.
4. Add 2.0 to 3.0 mL of 0.25% (w/v) trypsin-0.53 mM EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 15 to 20 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.
5. Transfer cell suspension to a 15mL centrifuge tube and spin at approximately 250 x *g* for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 4 to 6 x 10^4 viable cells/cm^2 is recommended.
7. Incubate cultures at 37°C (5% CO_2).

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 106 cells/ml.
3. Dispense the cells in 1 ml aliquots into cryo storage vials to give 2.5 X 106 cells/vial.
4. Freeze the cells in a cryo freezing-container overnight at -80°C.
5. Next day, transfer the cell vials to a liquid nitrogen tank for long-term storage.

Data Example

A

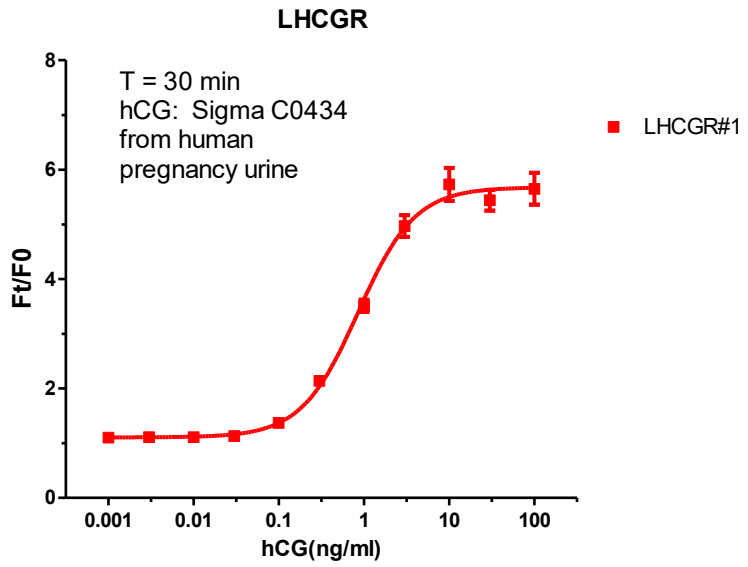


Figure 1. Response of ACT*One*™ LHCGR cell line & parental cell line to hCG.

ACTOne™ LHCGR cells were plated overnight in 20 µl culture medium on a 384 well BD 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 hours of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of hCG. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of hCG in ACT*One*™ LHCGR cell line. EC50 = 855 pM in the presence of PDE inhibitor Ro 20-1724.

Notice to Purchaser

1.This cell line is to be used for research purposes only. It may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of eEnzyme LLC.
2. Refer to the license agreement for details on the usage restrictions.