Human Recombinant Glucagon-like Peptide 1 Receptor Stable Cell Line

Cat. No. M00451 **Version 05282014**

I. INTRODUCTION

Catalog Number: M00451

Cell Line Name: CHO-K1/GLP1/Gα15

Gene Synonyms: GLP-1

Expressed Gene: Genbank Accession Number NM_002062; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for GLP1 receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

GLP1R binds specifically the glucagon-like peptide-1 (GLP1) and has much lower affinity for related peptides such as the gastric inhibitory polypeptide and glucagon. GLP1R is known to be expressed in pancreatic beta cells. Activated GLP1R stimulates the adenylyl cyclase pathway which results in increased insulin synthesis and release of insulin. Consequently, GLP1R has been suggested as a potential target for the treatment of diabetes. GLP1R is also expressed in the brain where it is involved in the control of appetite. Furthermore, mice which over express GLP1R display improved memory and learning.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae and M. capricolum) and one species Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by GLP-1 (7-37) in CHO-K1/GLP1/Gα15 cells

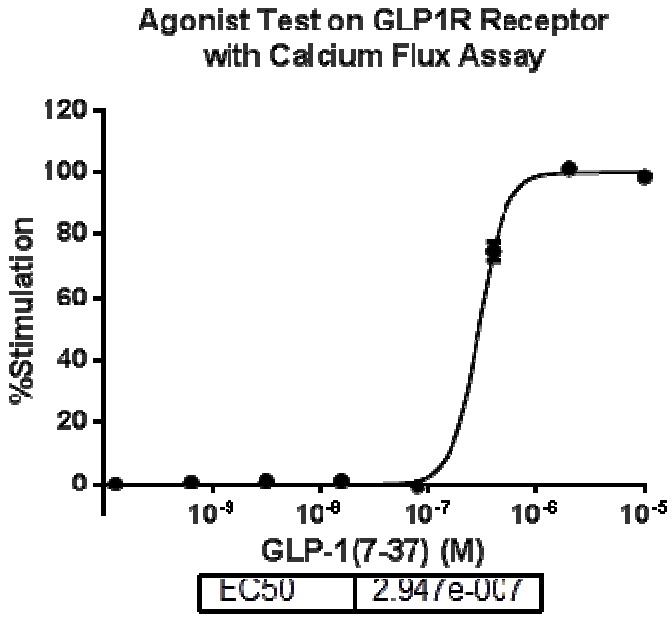

Figure 1. GLP-1 (7-37)-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GLP1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a GLP1 receptor agonist, GLP-1 (7-37). The intracellular calcium change was measured by FlexStation. The % stimulation was plotted against the log of the cumulative doses (5-fold dilution) of GLP-1 (7-37) (Mean ± SD, n = 2). The EC_{50} of GLP-1 (7-37) on GLP1 co-expressing with Gα15 in CHO-K1 cells was 295 nM. The S/B of GLP-1 (7-37) on GLP1 co-expressing with Gα15 in CHO-K1 cells was 18.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*HillSlope))
   X is the logarithm of concentration. Y is the response
   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
2. Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO_2.
7. In the following day, replace the cells with fresh medium contains antibiotic.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
   Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO_2.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

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3. Holst JJ (2004) Treatment of type 2 diabetes mellitus with agonists of the GLP-1 receptor or DPP-IV inhibitors. *Expert Opin Emerg Drugs* 9 (1): 155–66.
4. Kinzig KP, D'Alessio DA, Seeley RJ (2002) The diverse roles of specific GLP-1 receptors in the control of food intake and the response to visceral illness. *J. Neurosci.* 22 (23): 10470–6.
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