Human Recombinant Glucagon Receptor Stable Cell Line

Technical Manual No. TM0587 **Version 10132010**

I. Introduction

Catalog Number: M00422

Cell Line Name: HEK293/GCGR/Gα15

Gene Synonyms: GCGR; GGR; MGC138246

Expressed Gene: Genbank Accession Number NM_000160; no expressed tags

Host Cell: HEK293

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

Stability: 16 passages

Application: Functional assay for glucagon receptor

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

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 50 μg/ml Hygromycin B, 300 μg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

Glucagon regulates blood glucose via control of hepatic glycogenolysis and gluconeogenesis and via regulation of insulin release from the β cell. Pharmacological administration of glucagon increases blood glucose in normal and diabetic subjects, and produces positive inotropic and chronotropic cardiovascular effects, relaxation of smooth muscle in the gastrointestinal tract and stimulation of growth hormone secretion. The actions of glucagon are mediated via a single adenylate cyclase-coupled glucagon receptor that also couples to the phospholipase C-inositol phosphate (PLC-IP) pathway leading to Ca^{2+} release from intracellular stores.

§: 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.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Glucagon in HEK293/GCGR/Gα15 and HEK293 cells

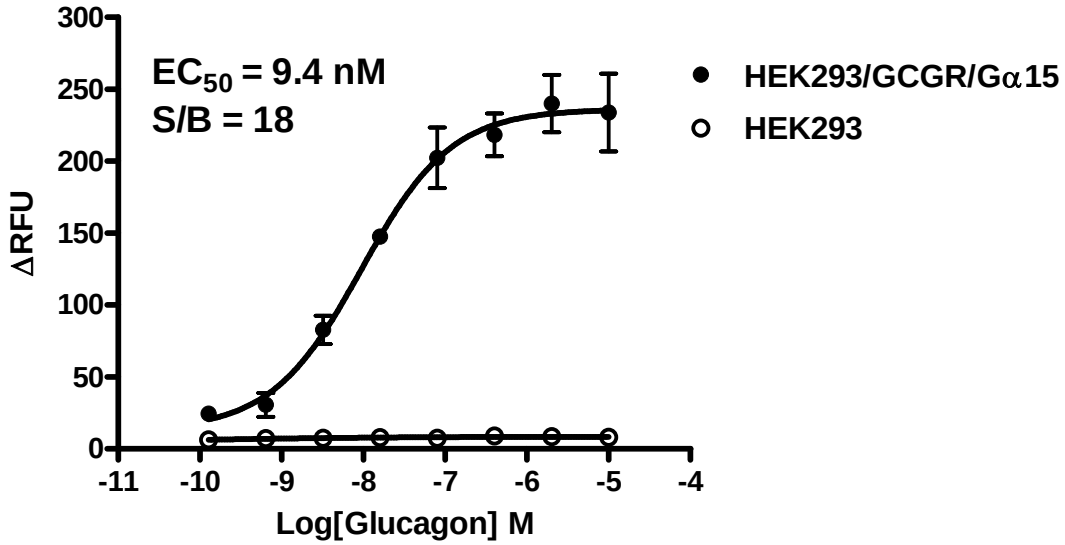

Figure 1. Glucagon-induced concentration-dependent stimulation of intracellular calcium mobilization in HEK293/GCGR/Gα15 and HEK293 cells. The cells were loaded with Calcium-4 prior to stimulation with a GCGR receptor agonist, Glucagon. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Glucagon (Mean ± SD, n = 2). The EC_{50} of Glucagon on GCGR co-expressing with Gα15 in HEK293 cells was 9.4 nM. The S/B of Glucagon on GCGR co-expressing with Gα15 in HEK293 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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Hygromycin B and G418 to concentrations of 50 μg/ml and 300 μg/ml respectively the following day.

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Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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

V. References

1. Moore, M. C. and Cherrington, A. D., Autoregulation of hepatic glucose production(1998). *Eur. J. Endocrinol*.. 138: 240-248
2. Samols, E. and Marks, V., Interrelationship of glucagon, insulin, and glucose: The insulinogenic effect of glucagon(1966). *Diabetes.* 15: 855-865
3. Muhlhauser, I. et al., Incidence and management of severe hypoglycemia in 434 adults with insulin-dependent diabetes mellitus(1985). *Diabetes Care.* 8: 268-273
4. White, C. M., A review of potential cardiovascular uses of intravenous glucagon administration (1999). *J. Clin. Pharmacol.* 39: 442-447
5. Wakelam, M. J. and Houslay, M. D., Activation of two signal-transduction systems in hepatocytes by glucagon(1986). *Nature.* 323: 68-71

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