Human Recombinant G-Protein Coupled Receptor 103 Stable Cell Line

Technical Manual No. TM0511 **Version 10132010**

I. Introduction

Catalog Number: M00321

Cell Line Name: CHO-K1/GPR103/ Gα15

Gene Synonyms: AQ27; GPR103; MGC149217; SP9155

Expressed Gene: Genbank Accession Number NM_198179; 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 GPR103 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, 100 μg/ml Hygromycin B, 400 μg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery.

II. Background

GPR103 is known as an orphan G protein-coupled receptor with reported expression in brain, heart, kidney, adrenal gland, retina, and testis. GPR103 mRNA has been reported to be highly expressed in the superficial layers of the entire spinal cord and a high density of 26RFa binding sites was observed in the superficial layers of the dorsal horn. QRFP binds and activates the human GPR103, as well as mouse GPR103A and GPR103B, with nanomolar affinities in transfected cells. Therefore, the current experiments investigated the effects of QRFP administration in rats and the effects of a high fat diet on prepro-QRFP mRNA and GPR103 receptor mRNA levels. 26RFa and QRFP are endogenous ligands of GPR103. An immunohistochemical study revealed that GPR103-like immunoreactivity (LI) was observed in the superficial layers of spinal dorsal horn, that QRFP-LI was observed in the dorsal root ganglion and that intrathecal 26RFa suppressed the expression of Fos-LI induced by paw formalin injection in the superficial layers of the spinal dorsal horn.

§: 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 Human 26RFa in CHO-K1/GPR103/Gα15 and CHO-K1/Gα15 cells

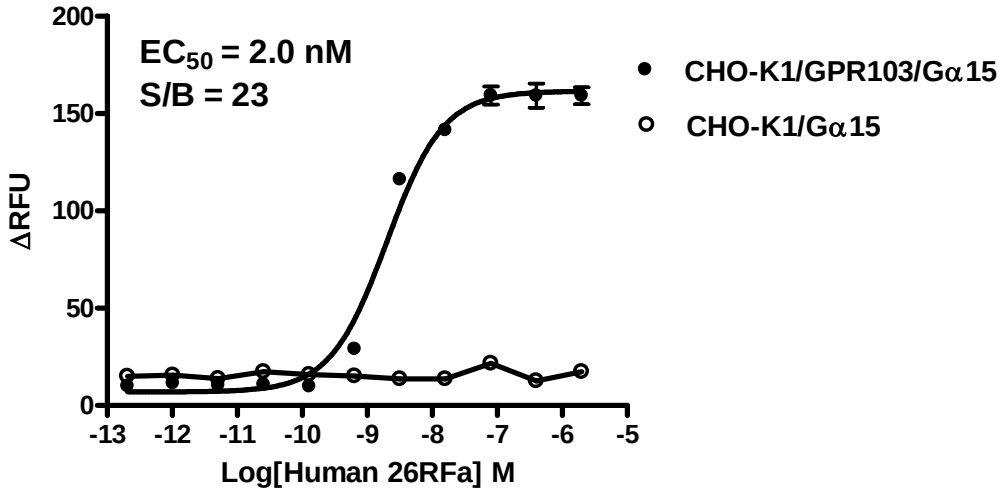

Figure 1. Human 26RFa-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GPR103/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a GPR103 receptor agonist, Human 26RFa. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Human 26RFa (Mean ± SD, n = 2). The EC_{50} of Human 26RFa on GPR103 co-expressing with Gα15 in CHO-K1 cells was 2.0 nM. The S/B of Human 26RFa on GPR103 co-expressing with Gα15 in CHO-K1 cells was 23.

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 the 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 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 100 μg/ml and 400 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 a 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 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. Baribault H, Danao J, Gupte J, *et al*. (2006) The G-protein-coupled receptor GPR103 regulates bone formation. *Mol. Cell. Biol.* 26(2):709-17.
2. Takayasu S, Sakurai T, Iwasaki S, *et al*. (2006) A neuropeptide ligand of the G protein-coupled receptor GPR103 regulates feeding, behavioral arousal, and blood pressure in mice. *Proc Natl Acad Sci U S A.* 103(19):7438-43
3. Yamamoto T, Wada T, Miyazaki R. (2008) Analgesic effects of intrathecally administered 26RFa, an intrinsic agonist for GPR103, on formalin test and carrageenan test in rats. *Neuroscience.* 157(1):214-22
4. Primeaux SD, Blackmon C, Barnes MJ, *et al*. (2008) Central administration of the RFamide peptides, QRFP-26 and QRFP-43, increases high fat food intake in rats. *Peptides.* 29(11):1994-2000

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