Human Recombinant Mas-related G Protein Coupled Receptor Member X2 Stable Cell Line

Cat. No. M00425 **Version 06092014**

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I. INTRODUCTION

Catalog Number: M00425
Cell Line Name: CHO-K1/MRGPRX2
Gene Synonyms: MRGPRX2; MRGX2
Expressed Gene: Genbank Accession Number NM_054030; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for MRGPRX2 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
Mycoplasma Status[§]: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

MRGX2 is a Gq- and Gi-coupled GPCR that binds to several peptides, including the neuropeptide cortistatin-14 and the proadrenomedullin N-terminal peptide (PAMP). It is probably involved in the function of nociceptive neurons and regulation of nociceptor function and/or development, including the sensation or modulation of pain. Dorsal root ganglia and adrenal chromaffin cells express MRGX2, and binding of these ligands to MRGX2 is thought to play a role in nociception and catecholamine release from the adrenal glands.

§: 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 Cortistatin 14 in CHO-K1/MRGPRX2 and CHO-K1 cells

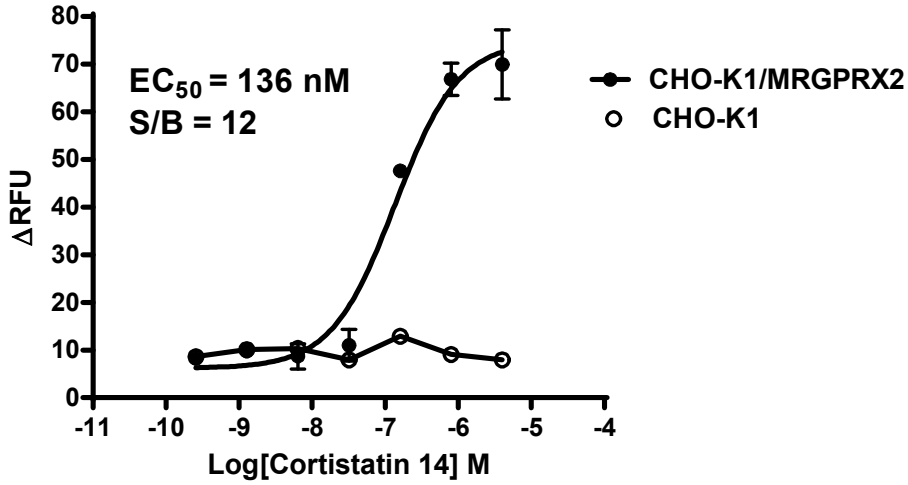

Figure 1. Cortistatin 14-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/MRGPRX2 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an MRGPRX2 receptor agonist, Cortistatin 14. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Cortistatin 14 (Mean ± SD, n = 2). The EC_{50} of Cortistatin 14 on MRGPRX2 in CHO-K1 cells was 136 nM. The S/B of Cortistatin 14 on MRGPEX2 in CHO-K1 cells was 12.

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 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

1. Robas, N., E. Mead, *et al.* (2003) MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *J Biol Chem*. 278(45): 44400-4.
2. Kamohara, M., A. Matsuo, *et al.* (2005) Identification of MrgX2 as a human G-proteincoupled receptor for proadrenomedullin N-terminal peptides. *Biochem Biophys Res Commun*. 330(4): 1146-52.

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