

Human Recombinant 5-HT4 Serotonin Receptor Stable Cell Line**Cat. No. M00444****Version 05292014**

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I. INTRODUCTION

Catalog Number: M00444

Cell Line Name: CHO-K1/5-HT4/G_{α15}

Gene Synonyms: HTR4, 5-HT4

Expressed Gene: Genbank Accession Number NM_000870; no expressed tags

Host Cell: CHO-K1/Gq α 15Quantity: Two vials of frozen cells (3×10⁶ per vial)

Stability: 16 passages

Application: Functional assay for 5-HT4 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, 400 µg/ml G418, 200 µg/ml HygromycinB

Plating medium: Ham's F12, 10% dialyzed FBS

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

5-HT4 is a human serotonin G protein-coupled receptor. Several studies suggested that intact 5-HT systems are important determinants of sensitivity and/or tolerance to ethanol-induced ataxia hypothermia. Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT receptor knock-out mice. Neuropsychiatric disorders such as anxiety, depression, migraine, vasospasm and epilepsy may involve different subtypes of the 5-HT receptor.

§: 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 5-Hydroxytryptamine (5-HT) in CHO-K1/5-HT4/Gqi5 and CHO-K1/Gqi5 cells

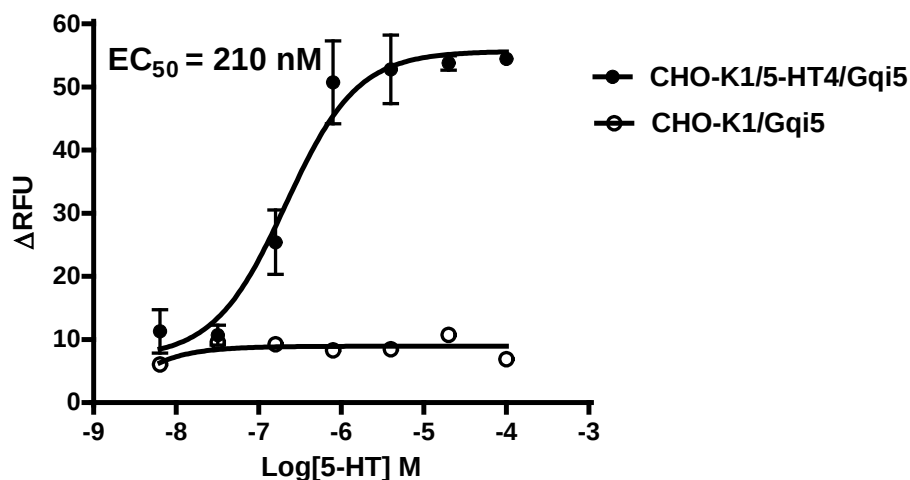


Figure 1. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/5-HT4/G_{α15} and CHO-K1/G_{α15} cells. The cells were loaded with Calcium-4 prior to stimulation with a 5-HT4 receptor agonist, 5-HT. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Histamine (Mean ± SD, n = 2). The EC₅₀ of 5-HT on 5-HT4 co-expressing with Gqi5 in CHO-K1 cells was 210 nM. The S/B of 5-HT on 5-HT4 co-expressing with Gqi5 in CHO-K1 cells was 8.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X) \cdot \text{HillSlope}})$$

X is the logarithm of concentration.
Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- 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.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.

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6. Grow the cells in incubator with 37°C, 5 %CO₂.
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 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Compan, V., Zhou, M., Grailhe, R., *et al.* (2004) Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT₄ receptor knock-out mice. *J. Neurosci.* 24(2):412-9.
2. Crabbe, J.C., Phillips, T.J., Feller, D.J., *et al.* (1996) Elevated alcohol consumption in null mutant mice lacking 5-HT_{1B} serotonin receptors. *Nat. Genet.* 14(1):98-101.
3. Oksenberg, D., Marsters, S.A., O'Dowd, B.F., *et al.* (1992) A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT_{1B} receptors. *Nature.* 360(6400):161-3.

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