

Human Recombinant H3 Histamine Receptor Stable Cell Line

Cat. No. M00331

Version 05282014

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I. INTRODUCTION

Catalog Number: M00331

Cell Line Name: CHO-K1/H3/G α 15

Gene Synonyms: HRH3; GPCR97; HH3R

Expressed Gene: Genbank Accession Number NM_007232; no expressed tags

Host Cell: CHO-K1/G α 15Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for H3 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 μ g/ml Hygromycin B, 400 μ g/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The histamine receptor H3 is a G $_i$ -coupled GPCR expressed in the thalamus, caudate nucleus, putamen, cerebellum, amygdala, substantia nigra, hippocampus, hypothalamus and cerebral cortex. Activation of H3 receptor in human nasal mucosa inhibits sympathetic vasoconstriction. Expression of human H3 receptor splice variants in CHO cells shows that a deletion in the 2nd transmembrane domain alters ligand binding and deletions in the 3rd intracellular loop result in signal transduction being abolished.

§: 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 Histamine in CHO-K1/H3/Gα15 and CHO-K1/Gα15 cells

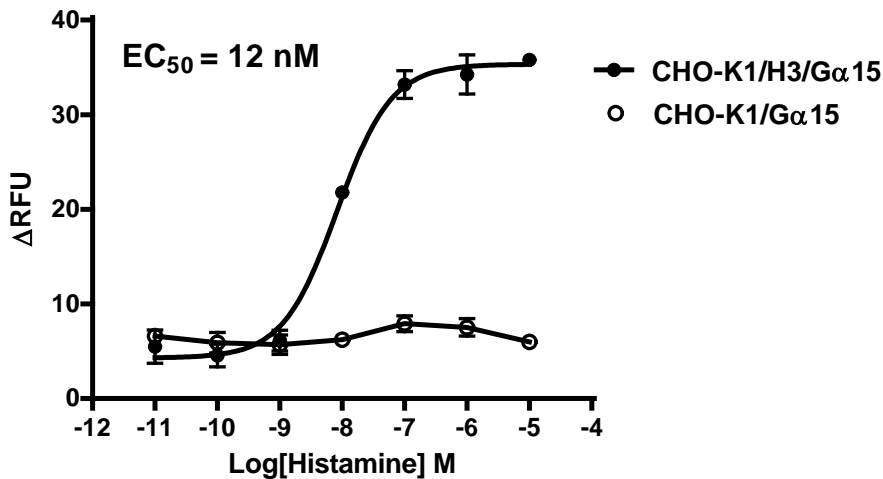


Figure 1. Histamine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/H3/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an H3 receptor agonist, Histamine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Histamine (Mean ± SD, n = 2). The EC₅₀ of Histamine on H3 co-expressing with Gα15 in CHO-K1 cells was 12 nM. The S/B of Histamine on H3 co-expressing with Gα15 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.

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. Clark EA *et al.* (1996) Hill SJ. Sensitivity of histamine H3 receptor agonist-stimulated [35S]GTP gamma[S] binding to pertussis toxin. *Eur J Pharmacol.* 296(2):223-5.
2. Cogé F *et al.* (2001) Genomic organization and characterization of splice variants of the human histamine H3 receptor. *Biochem J.* 355(Pt 2):279-88.
3. Varty LM *et al.* (2004) Activation of histamine H3 receptors in human nasal mucosa inhibits sympathetic vasoconstriction. *Eur J Pharmacol.* 484(1):83-9.

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