

Human Recombinant H4 Histamine Receptor Stable Cell Line

Cat. No. M00289

Version 05282014

I	Introduction	1
II	Background.....	1
III	Representative Data.....	2
IV	Thawing and Subculturing.....	2
V	References	3
	Limited Use License Agreement.....	4

I. INTRODUCTION

Catalog Number: M00289

Cell Line Name: CHO-K1/H4/Gα15

Gene Synonyms: GPRv53, HH4R, GPCR105, AXOR35, SP9144

Expressed Gene: Genbank Accession Number NM_021624; no expressed tags

Host Cell: CHO-K1

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

Stability Tested to: 16 passages

Application: Functional assay for H4 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

Histamine receptor family consists of four members, namely, H₁, H₂, H₃, and H₄. The histamine H₄ receptor is the most recently identified G protein-coupled histamine receptor that binds to several neuroactive drugs, including amitriptyline and clozapine. So far, H₄ receptors have been found only on haematopoietic cells. This signifies its important role as a drug target for the treatment of inflammatory diseases.

§: 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 Histamine in CHO-K1/H4/Gα15 and CHO-K1/Gα15 cells

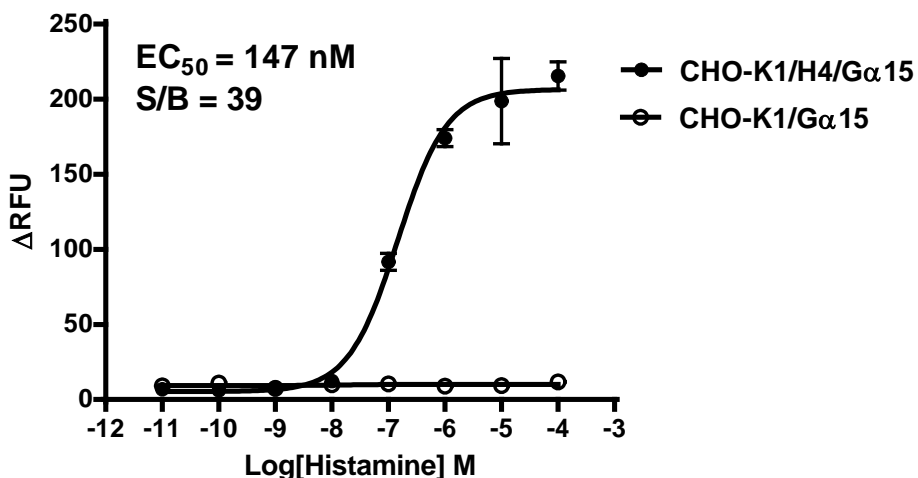


Figure 1. Histamine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/H4/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a H4 receptor agonist, Histamine. The intracellular calcium change was measured by FlexStation. 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 H4 co-expressing with Gα15 in CHO-K1 cells was 147 nM. The S/B of Histamine on H4 co-expressing with Gα15 in CHO-K1 cells was 39.

Notes:

1. 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 the response

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

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 %CO₂.
7. Add antibiotic in the following day.

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. Leurs R, Chazot PL, Shenton FC, Lim HD, de Esch IJ.Br. (2009) Molecular and biochemical pharmacology of the histamine H4 receptor. *J. Pharmacol.* 2009 May; 157(1):14-23.
2. Lim HD, van Rijn RM, Ling P, Bakker RA, Thurmond RL, Leurs R. (2005) Evaluation of histamine H1-, H2-, and H3-receptor ligands at the human histamine H4 receptor: identification of 4-methylhistamine as the first potent and selective H4 receptor agonist., *J. Pharmacol. Exp. Ther.* 2005 Sep; 314(3):1310-21.

GenScript USA Inc,

860 Centennial Ave.

Piscataway, NJ 08854

Toll-Free: 1-877-436-7274

Tel: 1-732-885-9188, Fax: 1-732-210-0262

Email: product@genscript.com

Web: <http://www.genscript.com>

For Research Use Only.

860 Centennial Ave., Piscataway, NJ 08854, USA

Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

- 1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
- 2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
- 3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 120 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.