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**Human Recombinant H3 Histamine Receptor Stable Cell Line****Cat. No. M00331****Version 07302020**

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**I. INTRODUCTION**

Catalog Number: M00331

Cell Line Name: CHO-K1/H3/Gα15

Gene Synonyms: HRH3; GPCR97; HH3R

Expressed Gene: Genbank Accession Number NM\_007232; no expressed tags

Host Cell: CHO-K1/Gα15

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1×10<sup>6</sup> per vial)

Stability: More than 16 passages

Application: Functional assay for H3 receptor (Calcium flux assay)

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS

Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen), 400 µg/ml G418 (Cat. #10131-035, Gibco)

Mycoplasma Status: Negative\*

Storage: Liquid nitrogen immediately upon receipt

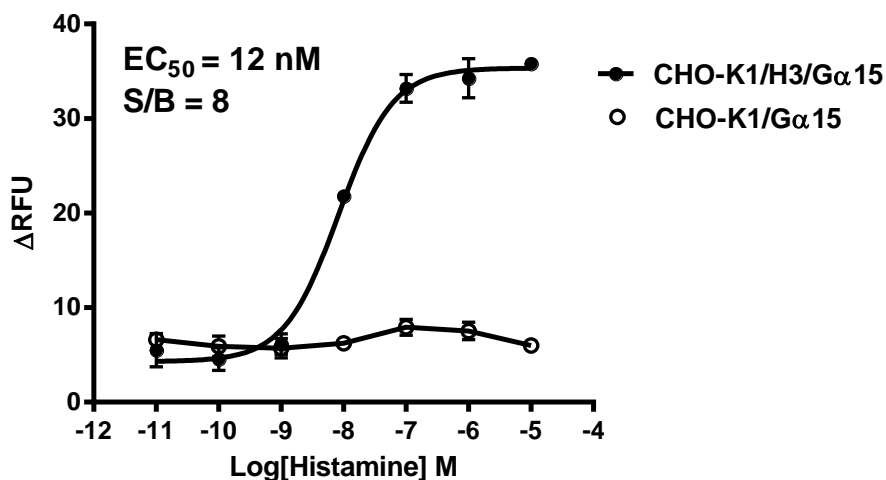
**II. BACKGROUND**

The histamine receptor H3 is a G<sub>i</sub>-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.

\* The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

### III. REPRESENTATIVE DATA

Calcium mobilization assay:



**Figure 1.** Histamine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/H3/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist histamine. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of Histamine (Mean ± SD, n = 2). The EC<sub>50</sub> of Histamine on this cell was 12 nM.

#### Notes:

- EC<sub>50</sub> 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

- 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.
- Grow the cells in incubator with 37°C, 5 %CO<sub>2</sub>.
- Add antibiotic in the following day.

#### Sub-culturing Protocol

- Remove the culture medium from cells.
- 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<sub>2</sub>.

Subcultivation Ratio: 1:3 to 1:8

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.

**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](mailto:product@genscript.com)  
Web: <http://www.genscript.com>  
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