Human Recombinant H2 Histamine Receptor Stable Cell Line

Technical Manual No. TM0446 **Version 06042010**

I. Introduction

Catalog Number: M00306

Cell Line Name: CHO-K1/H2/Gα15

Gene Synonyms: HRH2, DDX15, DBP1, H2R

Expressed Gene: Genbank Accession Number NM_022304; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: 2 vials (3×10^6 per vial) frozen cells

Stability: 16 passages

Application: Functional assay for H2 receptor

Freeze Medium: 45% culture medium,45% FBS, 10% DMSO

Complete Culture Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery.

II. Background

Histamine is a ubiquitous messenger molecule released from mast cells, enterochromaffin-like cells, and neurons. Its various actions are mediated by histamine receptors H1, H2, H3, and H4. Specifically, the histamine receptor H2 belongs to the rhodopsin-like family of G protein-coupled receptors. As an integral membrane protein, it stimulates gastric acid secretion and regulates gastrointestinal motility and intestinal secretion and is thought to be involved in regulating cell growth and differentiation.

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III. Representative Data

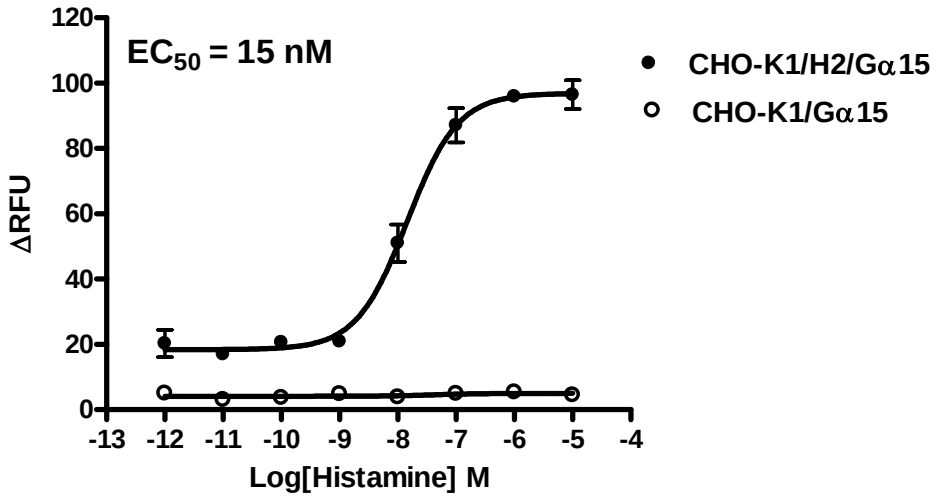

Figure Intracellular calcium response from CHO-K1/Gα15 cells stably expressing human H2 histamine receptors and from untransfected control cells. Cells were loaded with Calcium-4 then stimulated with the indicated concentrations of histamine. Calcium responses were recorded on a FlexStation plate reader. Data represent the average +/- standard deviation of triplicate determinations.

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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 2 ml of the cell suspension per well in a 6 well-plate.
6. Add Hygromycin B and Zeocin to concentrations of 100 µg/ml and 200 µg/ml respectively the following day.

Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in complete growth medium with Hygromycin B and Zeocin and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. References

1. Vannier E, Dinarello CA (1994) Histamine enhances interleukin (IL)-1-induced IL-6 gene expression and protein synthesis via H2 receptors in peripheral blood mononuclear cells. *J. Biol. Chem*. 269 (13): 9952–6.

2. Smit MJ, Timmerman H, Alewijnse AE, *et al*. (1995) Visualization of agonist-induced internalization of histamine H2 receptors. *Biochem. Biophys. Res. Commun*. 214 (3): 1138–45.

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