I. Introduction

Diverse physiological effects of histamine are mediated through cell surface G protein-coupled histamine receptors. To date, four subtypes of histamine receptors have been cloned: H1, H2, H3 and H4. The histamine H2 receptor (HRH2) couples to stimulatory Gs and induces activation of adenyl cyclase with subsequent increase of cAMP and PKA. In addition, recent work indicates that H2 receptor can also evoke the phosphoinositide signaling cascade. H2 receptor has been reported to be expressed in bone marrow, brain, heart, lymph node, nose, placenta, spinal cord, and stomach. The H2 receptors play important role in stimulating gastric secretion, gastrointestinal motility and intestinal secretion. Hence, H2-antagonists constitute a possible treatment of gastroduodenal ulcer. This manual describes establishment of a cell line and a protocol of pharmacologically validated human histamine H2 receptor (Genebank Accession Number: NM_022304).

II. Background:

- Catalog Number: M00132
- Cell Line Name: HEK/H2R/CRE/Luc
- Description:
  The HUMAN H2/HRH2 is amplified by PCR using a high fidelity enzyme and subcloned into the pcDNA3.1/hygro(+) mammalian expression vector. The full-length ORF has been confirmed by sequencing. The H2R reporter cell line was created by transfection of pcDNA3.1/H2R in a parental cell line, HEK293/CRE/Luciferase. The transfected cells were stably selected by 100 µg/ml Hygromycin. Single cell clones with high H2R inducibility and low luciferase background were isolated using ring cloning and serial dilution. The clones with largest dynamic range in luciferase were chosen for pharmacological and stability studies.
- Function: Cell based, functional assay for H2R receptor
- Quantity: 2 vial (2 x 10^6) frozen cells
- Passage Number froze: 2
- Stability Tested to: 16
- Host Cell: HEK 293
- Cell phenotype: Adherent/epithelial
- Mycoplasma: Negative
- Recommended Storage: Liquid nitrogen upon receiving.
- Propagation Medium: DMEM, 10% FBS, 150 µg/ml Hygromycin.
III. Representative Data

Histamine Dose Response of H2R/CRE/Luc Cell Line

\[ EC_{50} = 15.6 \text{ nM} \]

1. Cells are lightly trypsinized and seeded into 96-well plate at 25,000 cells/well (100 μl per well) in complete culture medium.
2. Culture cells in 5% CO2 at 37°C for 24 hours (at least overnight)
3. Add 10 μl 10X stimulation reagent into each well, being careful to not dislodge attached cells. Incubate cells in 37°C /CO2 for 6 hours.
4. To each well, add 100 μl of Bright-Glo Assay Reagent (equilibrated to room temperature 20-25°C), mix.
5. Transfer to a solid white 96-well plate (No transfer needed if the cells were incubated in a TC-treated clear bottom white plate).
6. Read luminescence with a luminescence plate reader at least two minutes after the reagent loading.
7. Collect data.

Attention: 3-5 steps should be done at room temperature, 25°C. All data should be collected in less than 15 minutes after reagent loading.

IV. Thawing and Subculturing

Cell Culture Conditions

Complete Culture Medium:
DMEM: 90%, FBS: 10%, L-glutamine 2.0 mM, Amp 100 μg/ml, Strep 100 μg/ml, Hygromycin 50 μg/ml

Thawing Cells:
1. Quickly thaw frozen cells in a 37°C water bath with a continuous agitation.
2. Using a 1 ml pipette, slowly pipet the cells up and down 5 times and add, drop by drop, to a 15 ml centrifuge tube containing 5 ml of fresh prewarmed complete DMEM medium. Then centrifuge at 1,000 rpm for 5 minutes.
3. Discard the supernatant medium and resuspend the cell pellet in 5 ml of fresh prewarmed complete DMEM medium. Transfer cells to a T25 flask and incubate at 37°C with 5% CO₂ until the cells
reach >90% confluence. The recovery rate for frozen cells is usually 90% or above.

**Subculturing:**
When the cells reach confluence, they need split. This cell line is normally split twice weekly at 1:8 to 1:15 dilutions.

1. Carefully aspirate all the media, gently rinse the cell layer with appropriate amount of 0.2% trypsin-EDTA, and aspirate it off.
2. Wait for about 1-3 minutes; dislodge the cells by gently tapping the sides of flask or dish.
3. Resuspend cells with appropriate amount of complete DMEM medium, and split cells as desired.

**Changing Medium:**
This is normally done every other day.

1. Gently aspirate off medium.
2. Transfer fresh warm complete DMEM medium (37°C) into a flask (5 ml for T25 and 10 ml for T75)

**Freezing Cells:**

1. Repeat steps 1-3 of Subculturing section.
2. Centrifuge down the cells at 1,000 rpm for 5 min.
3. Aspirate off the supernatant and resuspend the cells in fresh freezing medium at a density of 2-3 × 10^6 cells/ml. Add 1 ml cells per Cryogenic Vial.
4. Put the Cryogenic Vial of cells into Cryo Freezing Container, followed by transferring the container into -80°C and staying overnight.
5. Transfer Cryogenic Vial into liquid nitrogen (-196°C).

**Reagents & Consumables:**

1. DMEM: Dulbecco’s Modified Eagle Medium powder, high glucose (Gibco BRL, Cat #12100-046)
2. FBS: Fetal Bovine Serum (Hyclone, Cat #CH30160.03)
3. L-Glutamine: 200 mM (Gibco BRL, Cat # 25030-081)
4. Ampicillin: 50 mg/ml (Sigma A-9518)
5. Streptomycin Sulfate: 50 mg/ml (Gibco BRL, Cat # 11860-038)
6. Hygromycin B in PBS, 50 mg/ml (Invitrogen, Cat #10687-010)
7. Trypsin: 1:250 rom Bovine Pancreas (Gibco BRL, Cat # 27250016)
8. DMSO: dimethyl sulfoxide (Sigma, Cat #D8418)
9. Hepes: Sigma Cat #H-3375
10. Bright-Glo Assay Reagent (Promega E2620)
11. Histamine: Sigma Cat #H7125-1G
12. 96 Well White Round Bottom Polypropylene Not Treated Microplate (Corning, 3355)
13. 96 Well Flat Clear Bottom White Polystyrene TC-Treated Microplates (Corning, #3610)

**Media and Solutions:**

1. PBS (for preparation of 500 ml)
   1) KCl: 0.1 g
2) KH₂PO₄: 0.1 g
3) NaCl: 4.0 g
4) Na₂HPO₄·12H₂O: 1.4425 g

Dissolve the above components in double-distilled water (ddH₂O) and adjust pH to 7.4 with 0.1 N NaOH. Add ddH₂O to the final volume of 500 ml. Autoclave and store at 4°C.

2. Trypsin-EDTA (for preparation of 100 ml)
   1) Trypsin: 0.25 g
   2) 2%EDTA: 2 ml
   3) PBS: 98 ml

Dissolve trypsin in 2%EDTA and PBS completely; sterilize the solution by passing through a 0.20 um membrane filter; store at 4°C.

3. Culture medium (for preparation of 1 L)
   1) Measure out 950 ml distilled water to dissolve the media components with gentle stirring until the solution becomes clear.
   2) Add NaHCO₃ 3.7 g for high glucose DMEM
   3) Adjust pH of medium to 0.2-0.3 below the desired final working pH (using 1 N NaOH or 1 N HCL is recommended). Add slowly with stirring.
   4) Dilute to 1 liter with ddH₂O.
   5) Sterilize the medium immediately using the method of membrane filtration. Store at 4°C

4. Complete DMEM medium
   1) DMEM: 90%, FBS: 10%
   2) Supplements: L-glutamine 2.0 mM

5. Freezing Medium
   1) DMEM: 70%, FBS: 20%
   2) Supplements: dimethyl sulphoxide (DMSO) 10%

6. Ampicillin/Streptomycin 50 mg/ml

Dissolve 1 g Ampicillin or Streptomycin in 20 ml ddH₂O and sterilize the solution by membrane filtration using 0.20 μm filter. Aliquot and store at 4°C for short-term conservation and -20°C for long term conservation.

V. References
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.