

Human Histamine H4 Receptor Stable Cell Line

Technical Manual No. TM0311

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I	Introduction	1
II	Cell Line Information.....	1
III	Histamine Dose Response of HEK/H4R/Gα16 Cell Lin and Assay Procedure	2
IV	References	3
V	Appendix	3
	User Agreement (Label License).....	6

I. Introduction

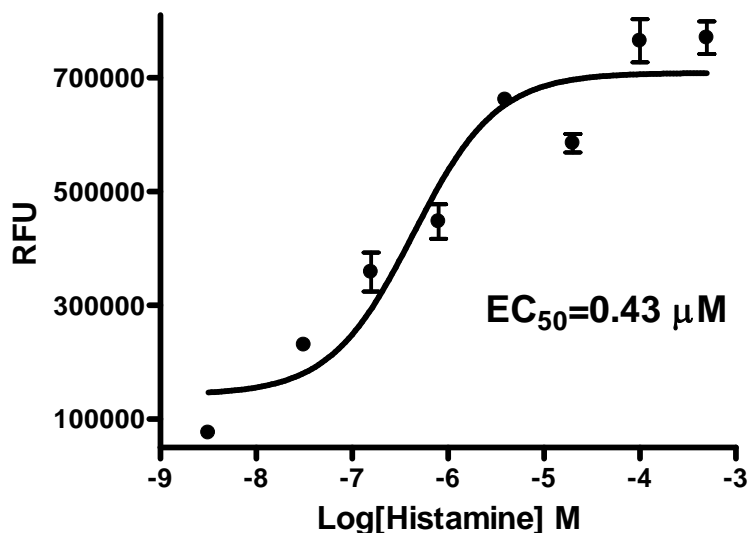
The diverse physiological effects of histamine are mediated through cell surface G protein-coupled histamine receptors. To date, four subtypes of histamine receptors have been cloned: H1, H2, H3, and H4. The histamine H4 receptor (HRH4) couples to stimulatory Gi and induces inhibition of adenylyl cyclase with subsequent decrease of cAMP and PKA. In addition, recent work indicates that the introduction of promiscuous G protein (Gα16) allows H4 receptor to mediate agonist-induced calcium ion mobilization. Expression of the H4 receptor seems to be limited to cells of hematopoietic lineage. In particular, H4 receptor expression has been observed in eosinophils, T cells, dendritic cells, basophils, and mast cells. Very little is known about the actual biological function of the H4 receptor. The H4 receptor can mediate chemotaxis and calcium influx in mast cells and eosinophils, both of which are important effector cells in the allergic response. H4 receptor possibly plays a role in allergic response and inflammation. This manual describes establishment of a cell line and a protocol of pharmacologically validated human histamine H4 receptor (Genebank Accession Number: NM_021624).

II. Cell Line Information

- Catalog Number: M00134
- Cell Line Name: HEK/H4R/Gα16
- Description:
 - The HUMAN H4/HRH4 is amplified by PCR using a high fidelity enzyme and subcloned into the pMT8 mammalian expression vector. The full-length ORF has been confirmed by sequencing. The H4R reporter cell line is created by transfection of pMT8/H4R in a parental cell line, HEK293/Gα16. The transfected cells are stably selected by 3 µg/ml puromycin. Single cell clones with high H4R inducibility and low background are isolated using ring cloning and serial dilution. The clones with largest dynamic range in calcium assay are chosen for pharmacological and stability studies.
- Function: Cell based, functional assay for H4R receptor
- Quantity: 2 vials (2-3 x 10⁶) frozen cells
- Passage Number shipped: 2
- Stability Tested to: 20
- Host Cell: HEK 293
- Cell phenotype: Adherent/epithelial
- Mycoplasma: Negative
- Recommended Storage: Liquid nitrogen upon receiving.
- Propagation Medium: DMEM, 10% FBS, 3 µg/ml puromycin.

III. Histamine Dose Response of HEK/H4R/Gα16 Cell Lin and Assay Procedure

Dose Response of HEK293/H4R/Gα16 Cell Line



1. Cells are seeded at a density of 3×10^4 to 5×10^4 cells/well in a 96-well black plate, 12-24 hours before running the assay.
2. The day of the assay, cells should be 100% confluent. Remove cell plates from the incubator. Do not remove the supernatant. Add an equal volume of Fluo3 Loading Buffer to each well (100 μL per well for 96-well plates, 25 μL per well for 384 well plates)
3. Incubate the plate at 37°C in the dark for 1 hr.
4. Prepare agonist addition plates in advance of assay. To a 96-well plate, add 10X work concentration of agonist compound in Calcium Assay solution A, and add 25 μl/well to cell plate.
5. Read with FlexStation or FLIPR using the specified settings and save data. The assay should be completed within 3 to 5 minutes after addition, but we recommend collecting data for a minimum of 6 minutes during assay development. Usually 90 seconds are enough.

Instrument Optical Parameters	
Excitation wavelength (nm)	485
Emission wavelength (nm)	525
Emission cut-off (nm)	515

IV. References

1. Changlu Liu, *et al.* Cloning and Pharmacological Characterization of a Fourth Histamine Receptor (H4) Expressed in Bone Marrow. *Mol Pharmacol*, 2001, 59:420-426.
2. Claudia L H, *et al.* Histamine H4 receptor Mediates Chemotaxis and Calcium Mobilization of Mast cells. *JPET*, 2003, 305:1212-1221.
3. Ping Ling *et al.* Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *British Journal of Pharmacology*, 2004, 142:161-171.

V. Appendix

Cell Culture Conditions

Note: *This clone often grows as clumps. It is necessary to separate cells into single cell suspensions whenever the cells are passaged or plated. MATRIGEL Matrix-coated flasks or plates are recommended for healthier cells.*

Complete Culture Medium:

DMEM: 90%, FBS: 10%, L-glutamine 2.0 mM, Amp 100 µg/ml, Strep 100 µg/ml, puromycin 3 µg/ml

Freezing Medium:

20% of fetal bovine serum and 10% dimethyl sulfoxide (DMSO)

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 $2-3 \times 10^6$ 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. Puromycin (Sigma, Cat #J593)
7. Trypsin: 1:250 rom Bovine Pancreas (Gibco BRL, Cat # 27250016)
8. MATRIGEL: BD Bioscience Cat# 354230
9. DMSO: dimethyl sulfoxide (Sigma, Cat #D8418)
10. Hepes: Sigma Cat #H-3375
11. Fluo-3, AM:(Molecular Probes 28B2-4)
12. Histamine: Sigma Cat #H7125-1G
13. 96 Well Plate: Costar, Cat# 3603, Blackwall/clear bottom, Polystyrene, sterilized.

Media and Solutions:

1. PBS (for preparation of 500 ml)

1) KCl:	0.1 g
2) KH ₂ PO ₄ :	0.1 g
3) NaCl:	4.0 g
4) Na ₂ HPO ₄ .12H ₂ O:	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 µm 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 NaHCO_3 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. 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.

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User Agreement (Label License) for Human Histamine H4 Receptor Cell Line

Catalog Number: M00134

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