HD Biosciences Co., Ltd

Product Description

HUMAN HISTAMINE H3 RECEPTOR CELL LINE

Product Number: CH2098-3

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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 H3 receptor (HRH3) 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 (Gα15) allowed H3 receptor to mediate agonist-induced calcium iron mobilization. H3 receptor has been reported to be expressed in the central nervous system .In rodents, The H3 receptors expression is observed in, for example, the cerebral cortex, hippocampal formation, amygdala, nucleus accumbens, globus pallidus, striatum and hypothalamus by autoradiography, immunohistochemistry or in situ hybridization. The H3 receptor is known to modulate various neurotransmitter systems in the brain. Hence, both H3-agonists and H3-antagonists might be of therapeutic use in the central nervous systems.

Using HDB's assay development technologies, a cell line and assay protocol was established and pharmacologically validated for H3R responsiveness to the histamine receptor agonists and antagonists, histamine, etc. The cell line is stable through at least passage 25 and demonstrates an approximately 3 fold dynamic range in the calcium assay thereby providing a good screening window. The H3R assay is ready for high throughput screening either as a primary or follow-up (selectivity screen) and can be used to identify agonists and antagonists.

Cell Line Information

- *Product Number:* CH2098-3
- *Description:*

  The HUMAN H3/HRH3 is amplified by PCR using a high fidelity enzyme and subcloned into the pcDNA3.1/ (+) mammalian expression vector. The full-length ORF has been confirmed by sequencing. The H3R reporter cell line was created by transfection of pcDNA3.1/H3R in an HDB parental cell line, HEK293/Gα15. The transfected cells were stably selected by 600 μg/ml G418. Single cell clones with high H3R inducibility and low background were isolated using ring cloning and serial dilution. The clones with largest dynamic range in calcium assay were chosen for pharmacological and stability studies.

- *Cell Line Name:* HEK/H3R/Gα15
- *Date Created:* 09/09/2005
- *Function:* Cell based, functional assay for H3R receptor
- *Quantity:* 2 vials (2-3 x 10^6) frozen cells
- *Passage Number Shipped:* 2
- *Host Cell:* HEK293
- *Cell phenotype:* Adherent/epithelial
- *Antibiotics Selection:* Hygromycin for parental cells and G418 for H3R cells
- *Freeze Medium:* Growth medium plus 20% FBS and 10% DMSO.
- *Plasmid:* huHRH3-pCDNA3.1/ (+)
- *Transfection:* Full-length human H3R cDNA (Genebank Accession Number: NM 007232)
- *Recommended Storage:* Liquid nitrogen upon receiving.
- *Propagation Medium:* DMEM, 10% FBS, 600 μg/ml G418, P/E.

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%

Supplements:

L-glutamine 2.0 mM

Amp 100 μg/ml

Strep 100 μg/ml

Medium for Stable Line Propagation:

Add 600 μg/ml G418 in complete culture medium.

Freezing Medium:

20-90% of fetal bovine serum and 10% dimethyl sulphoxide (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 1000 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% CO_2 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 minute; 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^{o}C) into a flask (5 ml for T25 and 10 ml for T75)

Freezing Cells:

1) Repeat the steps 1-3 0f 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 X 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^{o}C and staying overnight.
5) Transfer Cryogenic Vial into liquid nitrogen (-196^{o}C).

Assay Procedure

Calcium Assay of HEK/H3R/Gα15 Cells

1. Cells are seeded at a density of $3x10^4$ to $5x10^4$ 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, 25µL per well for 384 well plates)
3. Incubate the plate at 37^oC 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 HDB 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.

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

Results

H3R Inducibility of HEK/H3R/Gα15Cell Line

	Un-stimulated Control	Stimulated (histamine)
Background subtracted fluorescence read out (RFU)	17833	53292
Fold induction	1	3.0

Histamine Dose Response of HEK/H3R/Gα15 Cell Line

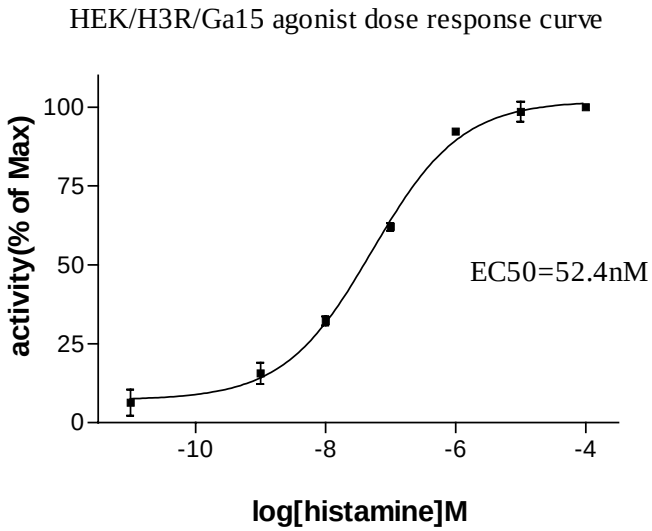

Fig. 1. Dose response of calcium activity as monitored with FlexStation plate reader upon treatment with ligand. Assay was done according to procedure described above. Data represent means ± SEM for duplicate samples. EC50 value for histamine dose response was determined using GraphPad Prism 4 software.

Clobenpropit Dose Response of HEK/H3R/Gα15 Cell Line

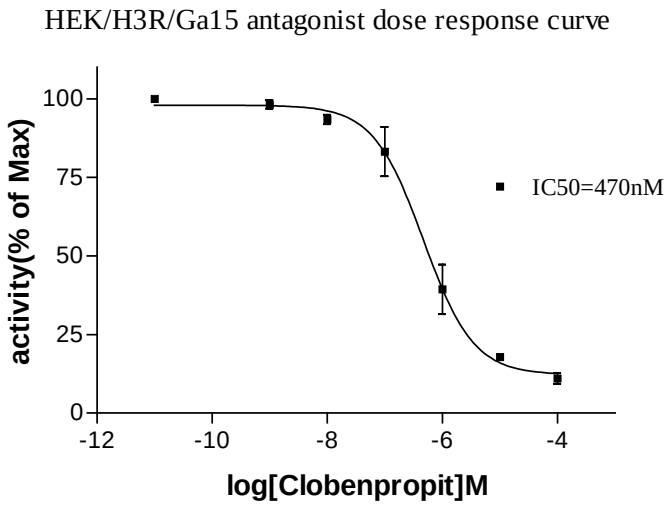

Fig. 2. Dose response of calcium activity as monitored with FlexStation plate reader upon treatment with ligand. Assay was done according to procedure described above. Data represent means ± SEM for duplicate samples. IC50 value for clobenpropit dose response was determined using GraphPad Prism 4 software.

Responses to HRs Subtype Specific Agonists

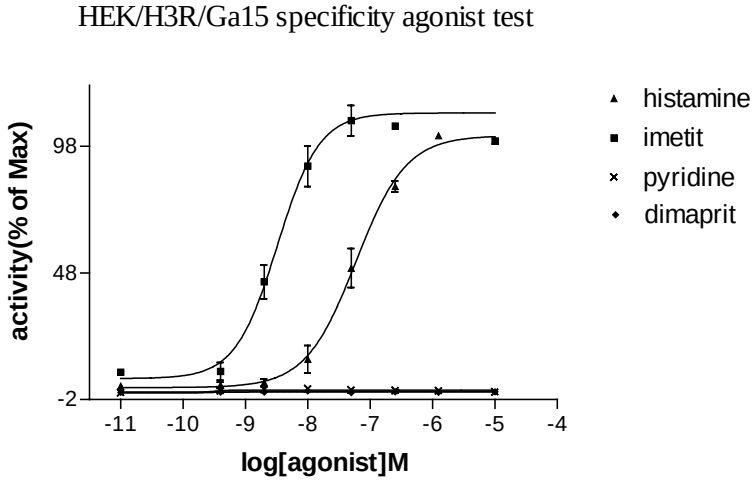

Fig. 3. HEK/H3R/Gα15 cell line was treated with different concentration of histamine, imetit, pyridine or dimaprit. The data shown were the average of one independent experiment performed in duplicate. Assay was done according to procedure described above.

The results indicate that HEK/H3R/Gα15 has very specific response to the treatment of subtype specific agonists and is H3R specific.

Responses to HRs Subtype Specific Antagonist

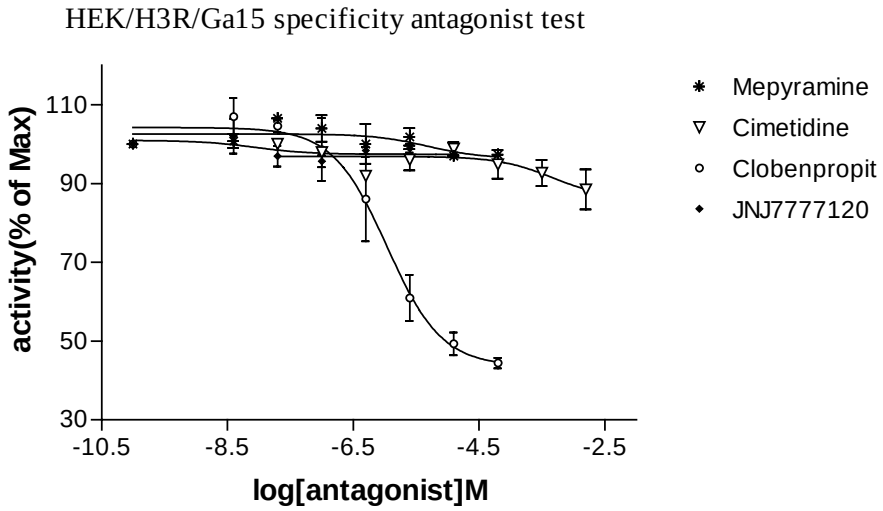

Fig. 4. HEK /H3R/Gα15 cell line was treated with different concentrations of mepyramine，cimetidine, clobenpropit or JNJ7777120. The data shown were the average of one independent experiment performed in duplicate. Assay was done according to procedure described above.

The results indicate that HEK/H3R/Gα15 has very specific response to the treatment of subtype specific antagonists and is H3R specific.

The Effect of DMSO to the Activity of HEK/H3R/Gα15 in Receptor Assay

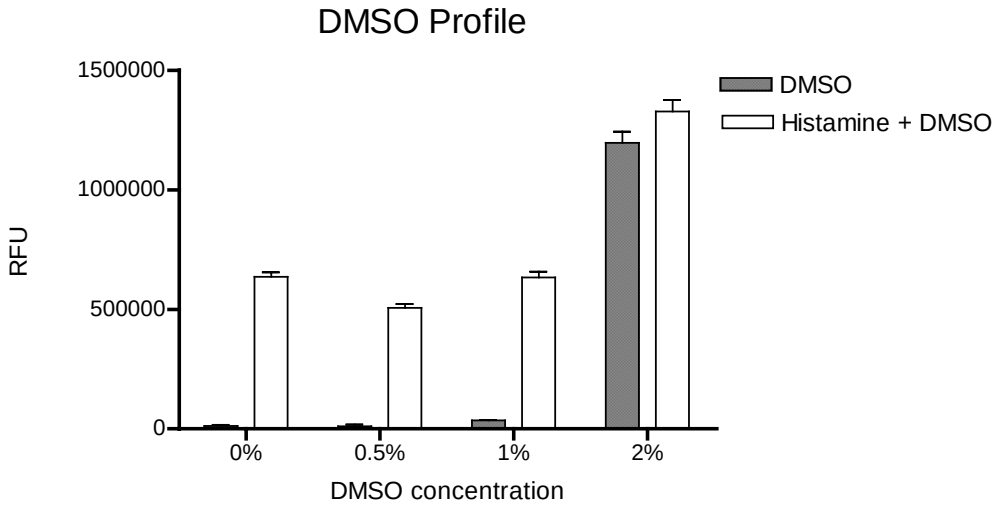

Fig. 5. Fluorescence activity was monitored with FlexStation plate reader upon treatment with ligand. HEK/H3R/Gα15 cell line was treated with histamine along with different concentrations of DMSO. Assay was done according to procedure described above. Data represent means ± SEM for duplicate samples.

The final concentration of DMSO below or equal to 1% does not induce any apparent calcium influx effect, while above 2% can generate significant calcium influx. Based on this result, we suggest that the final concentration of DMSO shall not surpass 1% in assay solution.

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The Determination of Optimal Number of Cells in the Assay Using HEK/H3R/Gα15

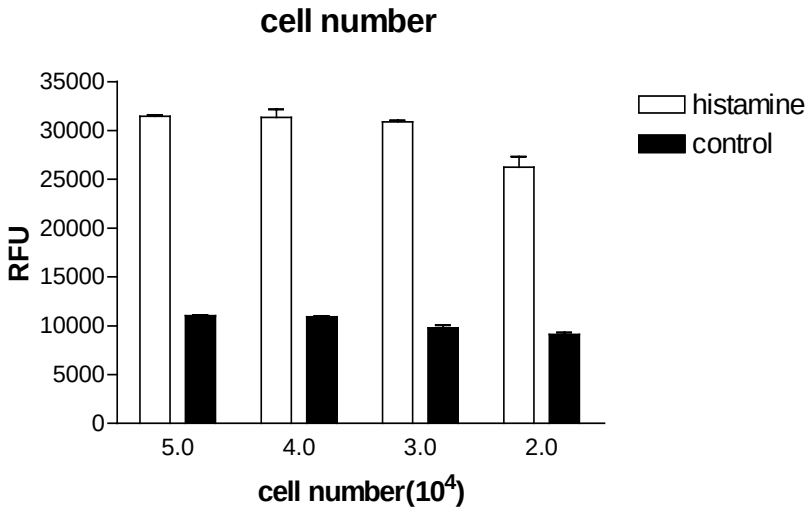


Fig. 6. Fluorescence activity was measured with FlexStation plate reader upon treatment with ligand. HEK/H3R/Gα15 cell was plated in 96-well plate with different cell number per well as indicated before the test. Assay was done according to procedure described above. Data represent means ± SEM for duplicate samples.

Based on this result, we suggest that 30,000-50,000 cells per well are used for the assay

HEK/H3R/Gα15 Cell Line Mycoplasma Detection

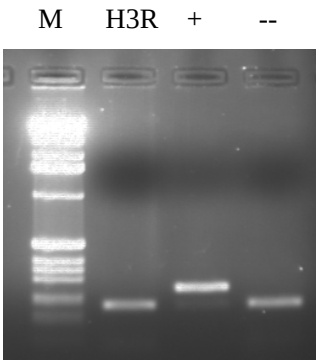


Fig. 7. Mycoplasma detection results with HEK/H3R/Gα15 cell line. PCR products run on 2% agarose gel.
Lane "H3R" referred to HEK/H3R/Gα15 cell line
Lane "+" referred to Mycoplasma positive control
Lane "-" referred to Mycoplasma negative control
Invitrogen 1kb ladder (Cat.No. 15615-016) was used as marker.

The result demonstrates that cell line is Mycoplasma free.

References

1. Leurs R, et al. The histamine H3 receptor: from gene cloning to H3 receptor drugs. 2005, Nat Rev Drug Discovery, 4:107-120.
2. S. J. Hill, C. R. Ganellin et. al. International Union of pharmacology. XIII. Classification of Histamine Receptors. 1997. Pharmacological Reviews. 49:253-278.
3. Kathleen M. Krueger, David G. Witte et.al. G protein-dependent pharmacology of histamine H3 receptor ligands: evidence for heterogeneous active state receptor conformations. 2005. JPET fast forward.

Appendix

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: 200mM (Gibco BRL, Cat # 25030-081)
4. Ampicillin: 50mg/ml (Sigma A-9518)
5. Streptomycin Sulfate: 50mg/ml (Gibco BRL, Cat # 11860-038)
6. G418 Sulfate, cell culture tested. (CALBIOCHEM, Cat #345810)
7. Trypsin: 1:250 rom Bovine Pancreas (Gibco BRL, Cat # 27250016)
8. MATRIGEL: BD Bioscience Cat# 354230
9. DMSO: dimethyl sulphoxide, for molecular biology (Sigma, Cat #D8418)
10. Hepes: Sigma Cat #H-3375
11. T25 flask: 25cm^2 cell culture flask (Corning Cat #430639)
12. 6 cm dish: (Orange, Cat # 2050200)
13. 6-well plate: (Corning Cat #3516)
14. Cryogenic Vial: (Corning Cat #430289)
15. Fluo-3, AM:(Molecular Probes 28B2-4)
16. Histamine: Sigma Cat #H7125-1G
17. 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_2PO_4: 0.1 g
    3) NaCl: 4.0 g
    4) $Na_2HPO_4.12H_2O$: 1.4425 g

Dissolve the above components in double-distilled water (ddH_2O) and adjust pH to 7.4 with 0.1 N NaOH. Add ddH_2O to the final volume of 500 ml. Autoclave and store at 4^oC.

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)

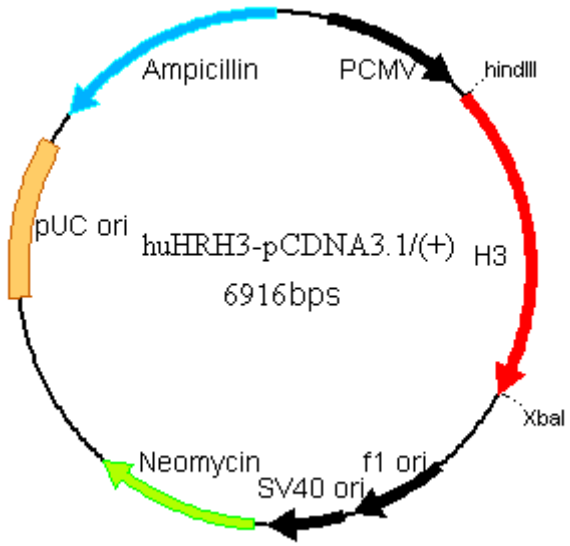
1) Measure out 950 ml distilled water to dissolve the media components with gentle stirring until the solution becomes clear.
2) Add $NaHCO_3$ 3.7 g for high glucose DMEM
3) Adjust pH of medium to 0.2-0.3 below the desired final working pH (using 1 N NaOH or 1 N HCL is recommended). Add slowly with stirring.
4) Dilute to 1 liter with ddH_2O.
5) Sterilize the medium immediately using the method of membrane filtration. Store at 4°C

4. Ampicillin/Streptomycin 50mg/ml

Dissolve 1g Ampicillin or Streptomycin in 20ml 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.

Map of huHRH3-pCDNA3.1/ (+)

Name：huHRH3-pCDNA3.1/ (+)
Insert gene：Homo sapiens histamine receptor H3, (HRH3)
Length of insert： 1338bp Sequence reference：Gene Bank NM_007232
Vector：pCDNA3.1/ (+) (Invitrogen) Insert site ： Hind Ⅲ (5') and Xba I (3')
Plasmid Map：



Comments for huHRH3-pCDNA3.1/ (+) **6916 nucleotides**
CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
BGH polyadenylation sequence: bases 2516-2740
f1 origin: bases 2786-3214
SV40 early promoter and origin: bases 3219-3588
Neomycin resistance gene (ORF): bases 3624-4418
SV40 early polyadenylation signal: bases 4592-4722
pUC origin: bases 5105-5775 (complementary strand)
Ampicillin resistance gene (bla): 5920-6780 (complementary strand)
ORF: bases 966-2300 (complementary strand)
bla promoter (P3): bases 6781-6879 (complementary strand)