

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT H₁ HISTAMINE RECEPTOR****CATALOG NUMBER:** HTS050C **QUANTITY:** 2 vials, 1 mL per vial**LOT NUMBER:** **CONCENTRATION:** 2 x 10⁶ cells/mL

BACKGROUND: Histamine exerts its biological effects through a family of four GPCRs. Stimulation of the H₁ histamine receptor causes smooth muscle contraction, induces vascular permeability, and stimulates catecholamine release from the adrenal medulla. H₁ is also widely expressed in brain, and activation of H₁ excites several types of neurons by blocking potassium conductance (Hill *et al.*, 1997). Chemicon's cloned human H₁-expressing cell line is made in the Chem-3 host, which supports high levels of recombinant H₁ expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between H₁ and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

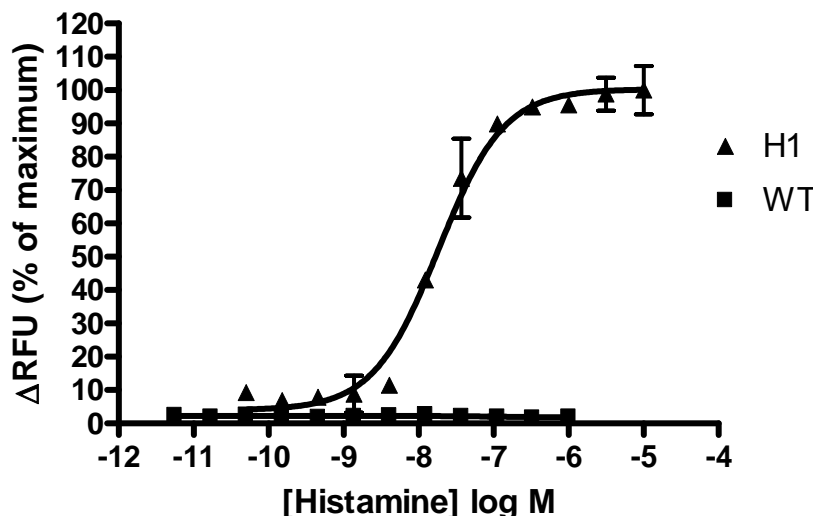


Figure 1. Calcium flux in H₁-expressing Chem-3 cell line induced by Histamine. H₁-expressing Chem-3 cells were loaded with Fluo-4 NW dye (Invitrogen) and calcium flux in response to histamine (10⁻⁵ to 10⁻¹¹ M) was determined in duplicate on a Molecular Devices FLIPR^{TETRA™}.

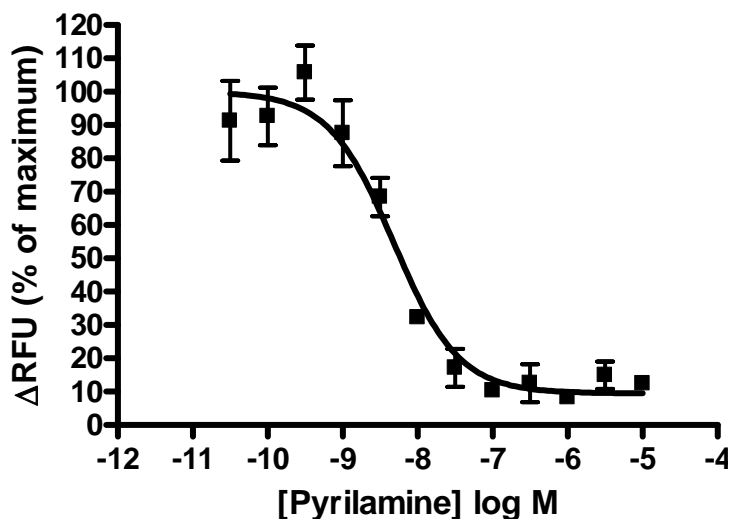


Figure 2. Assay for antagonist activity at H₁ by calcium flux assay. H₁-expressing Chem-3 cells were loaded with Fluo-4 and washed. Pylramine was added to the cells at the final concentration indicated, and incubated for 10 min at 37°C. Calcium flux in response to histamine (40 nM) was determined in duplicate on a Molecular Devices FLIPR^{TETRA™}.

SPECIFICATIONS: EC₅₀ for calcium mobilization by histamine: ~ 23 nM
IC₅₀ for pylramine with 2xEC₅₀ histamine: ~4.8 nM

HOST CELLS: Chem-3, a suspension cell line endogenously expressing the promiscuous G protein, G_{α15}, and lacking endogenous expression of histamine receptors.

TRANSFECTION: Full-length human HRH1 cDNA (Accession Number: NM_000861)

Cells are frozen at 2 x 10⁶ cells/mL in RPMI/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

PRESENTATION:

STORAGE/HANDLING:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a conical tube containing 10 mL Chem-3 Growth Media. Centrifuge at 200 x g for 5 min. Remove the supernatant and resuspend the cell pellet in 20 mL Chem-3 Growth Media. Transfer to a T75 flask and place the flask in a humidified incubator at 37°C with 5% CO₂.
3. Maintain the cells in suspension between 0.1 x 10⁶ and 1 x 10⁶ cells/mL. Cells are typically passaged 1:10 every 2-3 days, or 1:20 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator. Chem-3 cells tend to proliferate rapidly, so care should be taken not to allow them to become overconfluent.

4. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count cells. Centrifuge cells at 200 x g for 5 min. Resuspend cells at 2-5 x 10⁶ cells/mL in Chem-3 Freezing Media. Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at -70°C overnight. Store the vials in liquid nitrogen.
5. Cells should be passaged at least once after thawing prior to use in calcium flux assays.

MEDIA:

Chem-3 Growth Media:

RPMI-1640 containing 0.3 g/L L-glutamine, no pyruvate (Millipore SLM-140)
10% heat-inactivated FBS
100 U/ml each penicillin and streptomycin (from 100x stock, Millipore TMS-AB2-C)
1% Chem-3 Growth Supplement (Millipore catalog # HTSCHEM-3S)
G-418/Geneticin (600ug/mL)

Note: The Chem-3 Growth Supplement is required for growth of Chem-3 cell lines

Chem-3 Plating Media:

RPMI-1640 containing 0.3 g/L L-glutamine, no pyruvate
10% heat-inactivated FBS
100 U/ml each penicillin and streptomycin

Chem-3 Freezing Media:

RPMI-1640 containing 0.3 g/L L-glutamine, no pyruvate
20% heat-inactivated FBS
10% DMSO (cell culture grade)

**RECOMMENDATIONS
FOR CALCIUM ASSAY:**

Several calcium-sensing dyes are available that eliminate the need for washing. Any of these dyes may be used by the manufacturer's protocol, with the following recommendations for suspension cells such as Chem-3:

1. Cells should be passaged at least once after thawing, and should be in mid-log phase of growth at the time of the assay (0.8-1 x 10⁶ cells/mL). Propagate desired number of cells; 2 x 10⁵ cells/well are recommended for the assay. If the cell density exceeds 1 x 10⁶ cells/mL or the media is becoming yellow, passage the cells again and culture for at least one day before proceeding.
2. On the day of the assay, count cells and collect the desired number of cells. Centrifuge cells and resuspend at 2 x 10⁶ cells/ml in FLIPR assay buffer. Plate 50µL of cell suspension/well (96-well plate) and allow cells time to settle and equilibrate to new buffer environment at 37°C for at least one hour.
3. Prepare a 2x dye loading solution. After the cell incubation (step 2) is complete, add 50µL/well of the 2x dye loading solution for a final volume of 100µL. Incubate the cells at 37°C in the dark for 30 – 60 minutes.
4. For FLIPR ligand addition, set pipet tip height at 125 µL and dispense rate of 25 µL/sec. Hill SJ *et al.* (1997) International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev.* 49: 253-278.
For research use only; not for use as a diagnostic.

REFERENCES:

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HUMAN RECOMBINANT H₁ HISTAMINE RECEPTOR**

Product No. HTS050C

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