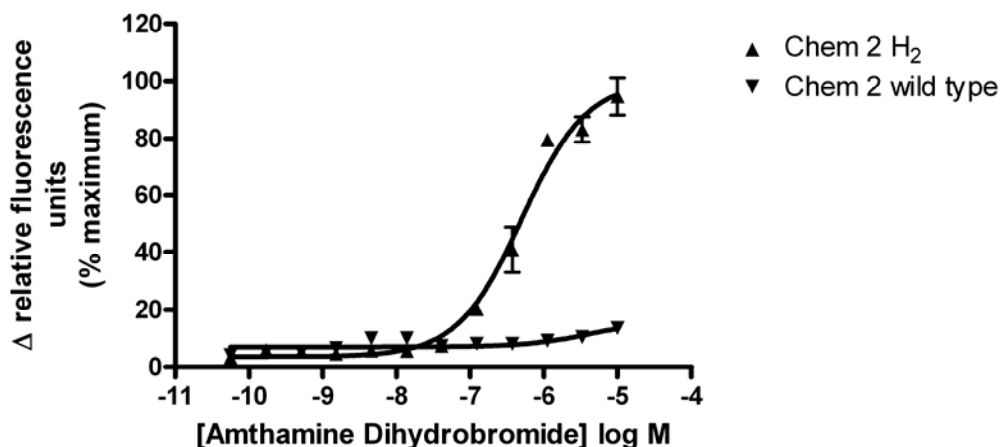


**ChemiScreen<sup>™</sup> CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT H<sub>2</sub> HISTAMINE RECEPTOR**

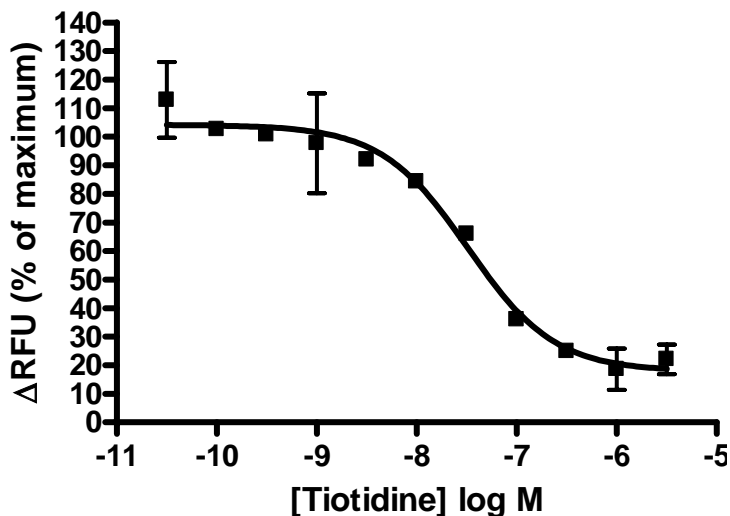
<b>CATALOG NUMBER:</b>	HTS086C	<b>QUANTITY:</b>	2 vials, 1 mL each
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/mL

**BACKGROUND:** The histamine H<sub>2</sub> receptor is a widely distributed G-protein coupled receptor, which is important in regulating a host of physiologic actions extending from gastric acid secretion to gastrointestinal motility (Del Valle and Gantz, 1997). Histamine mediated activation of its receptor leads to signaling through both adenylate cyclase and phosphoinositide /protein kinase C second messenger systems (Hill, 1990). Antagonists for this receptor have proven to be effective therapy for acid peptic disorders of the GI tract. Certain antagonists are used in the treatment of neuropsychiatric and neurological diseases e.g. schizophrenia, Alzheimer's disease and Parkinson's disease. Chemicon's cloned human H<sub>2</sub> expressing cell line is made in the Chem-2 host, which supports high levels of H<sub>2</sub> expression on the cell surface and contains high levels of the promiscuous G protein Gα16 to enhance coupling of the receptor to the calcium-signaling pathway. The cell line is an ideal tool for screening for antagonists of interaction between H<sub>2</sub> and its ligands.

**APPLICATIONS:** Calcium flux assay, ligand binding assays



**Figure 1.** Calcium flux in H<sub>2</sub>-expressing Chem-2 cell line. H<sub>2</sub>-expressing Chem-2 cells were loaded with Fluo-4 and calcium flux in response to Amthamine (10<sup>-5</sup> to 10<sup>-10</sup> M) was determined in triplicate on a Molecular Devices FLIPR<sup>TETRA™</sup>.



**Figure 2.** Assay for antagonist activity at H<sub>2</sub> by calcium flux assay. H<sub>2</sub>-expressing Chem-2 cells were loaded with Fluo-4. Tiotidine was added to the cells at the final concentration indicated, and incubated for 10 min at 37°C. Calcium flux in response to a 2x EC<sub>50</sub> dose of amthamine was determined in duplicate on a Molecular Devices FLIPR<sup>TETRA™</sup>.

SPECIFICATIONS: EC<sub>50</sub> for calcium mobilization by amthamine: ~ 503.7 nM  
IC<sub>50</sub> for tiotidine with 2x EC<sub>50</sub> concentration of amthamine: 31.5 nM

HOST CELLS: Chem-2, a suspension cell line expressing the promiscuous G-protein, G<sub>α16</sub>.

TRANSFECTION: Full-length human HRH2 cDNA encoding H<sub>2</sub> (Accession Number: NM\_022304)

**PRESENTATION:**

Cells are frozen at 2 x 10<sup>6</sup> cells/mL in 95% fetal bovine serum/5% DMSO. Cell line tests negative for mycoplasma.

**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 T75 containing 20 mL Chem-2 Plating Media (G418-free). Place the flask in a humidified incubator at 37°C with 5% CO<sub>2</sub> for 16-24 h to allow cells to recover from thawing prior to centrifugation.
3. To remove residual DMSO, centrifuge at 200 x g for 5 min. Remove the supernatant and resuspend the cell pellet in 20 mL Chem-2 Plating Media (G418-free). Transfer to a T75 flask and place the flask in a humidified incubator at 37°C with 5% CO<sub>2</sub>.
4. After 5-7 days, cells should be proliferating, and should be cultured in the presence of G418 to prevent loss of GPCR expression. Centrifuge at 200 x g for 5 min. Remove the supernatant and resuspend the cell pellet in 20 mL Chem-2 Growth

Media .

5. Maintain the cells between  $0.1 \times 10^6$  and  $1 \times 10^6$  cells/mL. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count cells. Centrifuge cells at  $200 \times g$  for 5 min. Resuspend cells at  $2-5 \times 10^6$  cells/mL in Chem-2 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  $-70^\circ\text{C}$  overnight. Store the vials in liquid nitrogen.
7. Cells should be passaged at least once after thawing prior to use in calcium flux assays.

**MEDIA:**

Chem-2 Growth Media:

RPMI-1640 with 25 mM HEPES and 2 mM L-glutamine (Millipore cat. # SLM-140)  
20% heat-inactivated FBS  
1x Pen-Strep  
Geneticin/G-418 (400ug/mL)

Chem-2 Plating Media:

RPMI-1640 with 25 mM HEPES and 2 mM L-glutamine (Millipore cat. # SLM-140)  
20% heat-inactivated FBS  
1x Pen-Strep

Chem-2 Freezing Media:

95% heat-inactivated FBS  
5% 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:

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 ( $0.8-1 \times 10^6$  cells/mL). Propagate desired number of cells;  $2 \times 10^5$  cells/well are recommended for the assay. If the cell density exceeds  $1 \times 10^6$  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  $2 \times 10^6$  cells/ml in FLIPR assay buffer. Plate  $50\mu\text{L}$  of cell suspension/well (96-well plate) and allow cells time to settle and equilibrate to new buffer environment at  $37^\circ\text{C}$  for at least one hour.
3. Prepare a 2x dye loading solution. After the cell incubation (step 2) is complete, add  $50\mu\text{L}$ /well of the 2x dye loading solution for a final volume of  $100\mu\text{L}$ . Incubate the cells at  $37^\circ\text{C}$  in the dark for 30 – 60 minutes.
4. For FLIPR ligand addition, set pipet tip height at  $125 \mu\text{L}$  and dispense rate of  $25 \mu\text{L}/\text{sec}$ .

**REFERENCES:**

Del Valle, J., and Gantz, I. (1997) Novel insights into histamine H2 receptor biology. *Am. J. Physiol.* **273**, G987–G996.

Hill, S. J. (1990) Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol. Rev.* **42**, 45–83.

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HUMAN RECOMBINANT H<sub>2</sub> HISTAMINE RECEPTOR**

**Product No. HTS086C**

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