

Human Recombinant 5-HT1A Serotonin Receptor Stable Cell Line**Cat. No. M00330****Version 05232014**

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I. INTRODUCTION

Catalog Number: M00330

Cell Line Name: CHO-K1/5-HT1A/Gα15

Gene Synonyms: HTR1A; 5-HT1A; 5HT1a;

Expressed Gene: Genbank Accession Number NM_000524; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for 5-HT1A receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Plating medium: Ham's F12, 10% dialyzed FBS

Culture Medium: Ham's F12, 10% FBS, 100 µg/ml Hygromycin B, 200 µg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The 5-Hydroxytryptamine receptor 1A (5-HT1A) is G_i-coupled GPCRs expressed in the cerebral cortex, hippocampus, septum, amygdala, and raphe nucleus, with lesser amounts in the basal ganglia and thalamus. Many functions of the central nervous system are influenced by 5-HT, including sleep, motor activity, sensory perception, arousal and appetite. 5-HT1A ligands may prove to be therapeutic in the treatment of various disorders such as depression, anxiety, and schizophrenia.

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§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritidis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by 5-HT in CHO-K1/5-HT1A/G α 15 and CHO-K1/G α 15 cells

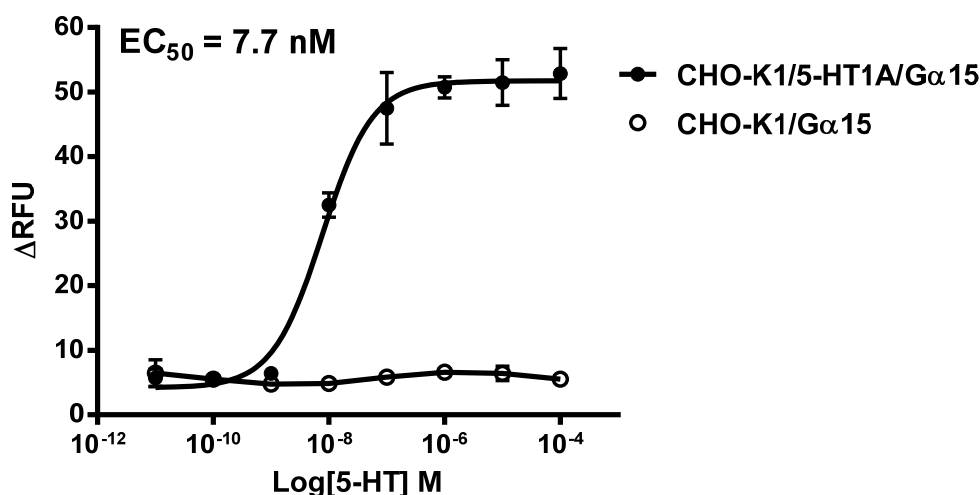


Figure 1. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/5-HT1A/G α 15 and CHO-K1/G α 15 cells. The cells were loaded with Calcium-4 prior to stimulation with a 5-HT1A receptor agonist, 5-HT. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of 5-HT (Mean $\pm$ SD, n = 2). The EC₅₀ of 5-HT on 5-HT1A co-expressing with G α 15 in CHO-K1 cells was 7.7 nM. The S/B of 5-HT on 5-HT1A co-expressing with G α 15 in CHO-K1 cells was 12.

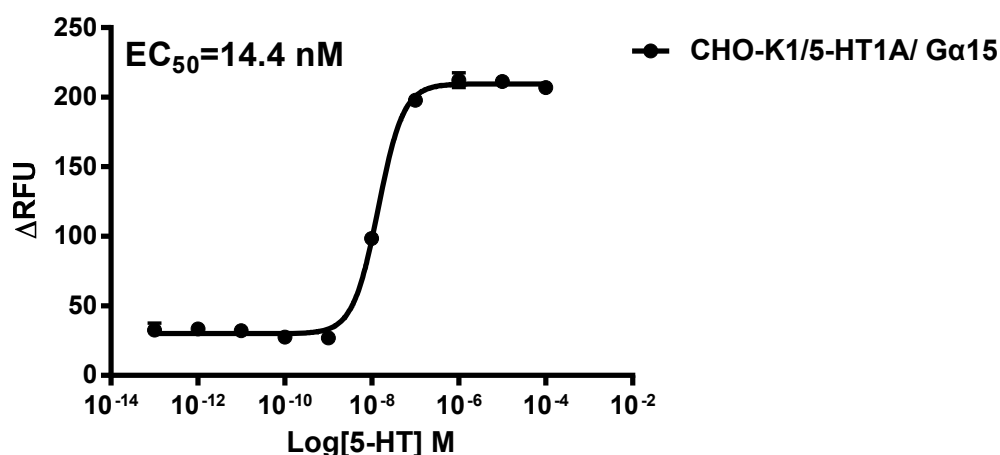


Figure 2. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/5-HT1A/G α 15 cells. The cells were loaded with Calcium-4 prior to stimulation with a 5-HT1A receptor agonist, 5-HT. The intracellular calcium change was measured by FLIPR^{tetra}. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of 5-HT (Mean $\pm$ SD, n = 2). The EC₅₀ value of 5-HT on 5-HT1A

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co-expressing with Ga15 in CHO-K1 cells was 14.4 nM. The S/B of 5-HT on 5-HT1A co-expressing with Ga15 in CHO-K1 cells was 7.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{Log}EC_{50} - X) * \text{HillSlope}))}$$

X is the logarithm of concentration. Y is the response
Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
2. Signal to background Ratio (S/B) = $\text{Top} / \text{Bottom}$

IV. RADIOLIGAND BINDING ASSAY

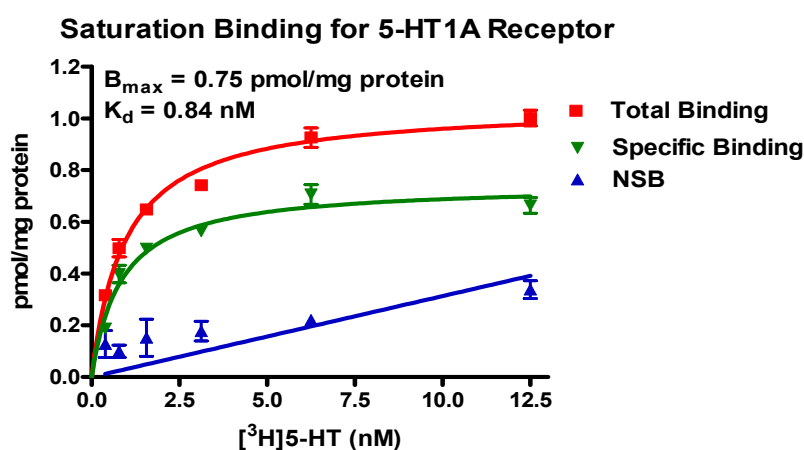


Figure 3. 10 μ g of membranes prepared from CHO-K1 cells stably expressing 5-HT1A receptors were incubated with indicated concentrations of [³H]5-HT in the absence (total binding) or presence of 1000-fold excess unlabeled Serotonin (nonspecific binding, NSB). Binding was terminated by rapid filtration. Specific binding was defined by subtracting NSB from total binding. Data were fit to one-site binding equation using a non-linear regression method.

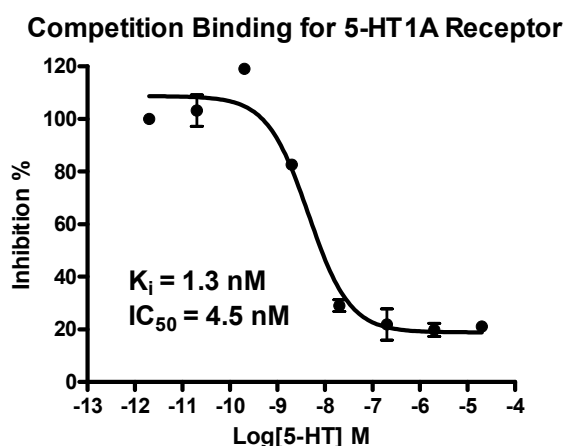


Figure 4. 10 μ g of membranes prepared from CHO-K1 cells stably expressing 5-HT1A receptors were incubated with

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indicated concentrations of Serotonin in the presence of 2 nM [³H]5-HT. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.

V. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO₂.
7. In the following day, replace the cells with fresh medium contains antibiotic.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

VI. REFERENCES

1. Hamon M *et al.* (1990) The main features of central 5-HT₁ receptors. *Neuropsychopharmacology*, 3(5-6):349-60.
2. Savitz J *et al.* (2009) 5-HT (1A) receptor function in major depressive disorder. *Prog Neurobiol*, 88(1):17-31.

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