

Human Recombinant 5-HT_{2B} Serotonin Receptor Stable Cell Line**Cat. No. M00249****Version 05292014**

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I. INTRODUCTION

Catalog Number: M00249

Cell Line Name: CHO-K1/5-HT_{2B}Gene Synonyms: 5-HT_{2B}; 5-HTR2B; HTR1C

Expressed Gene: Genbank Accession Number NM_000867; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10⁶ per vial)

Stability: 16 passages

Application: Functional assay for 5-HT_{2B} receptor

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

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

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

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

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

5-Hydroxytryptamine (5-HT, also commonly known as serotonin) is synthesized in enterochromaffin cells in the intestine and in serotonergic nerve terminals. In the periphery, 5-HT mediates gastrointestinal motility, platelet aggregation, and contraction of blood vessels. Many functions of the central nervous system are influenced by 5-HT, including sleep, motor activity, sensory perception, arousal and appetite. A family of 12 GPCRs and one ion channel mediate the biological effects of 5-HT (Hoyer et al., 1994). 5-HT_{2B} which couples to Gq/11 is expressed in embryonic and adult cardiovascular tissues, gut, and brain from the rat, mouse, and human species. 5-HT_{2B} receptors are responsible for many cardiovascular and central nervous system functions, such as blood vessel contraction, platelet shape changes, neuronal sensitization to tactile stimuli, and mediation of the hallucinogenic effects of phenylisopropylamin

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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. arthritis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

hallucinogens. It has also been shown to be required for heart development. GenScript's cloned human 5-HT2B-expressing cell line is generated in the CHO-K1 host.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by 5-HT in CHO-K1/5-HT2B and CHO-K1 cells

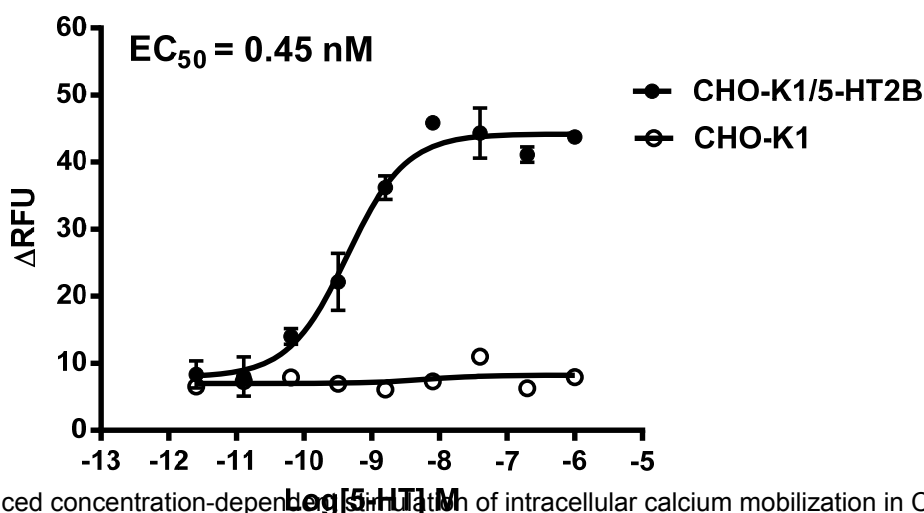


Figure 1. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/5-HT2B and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a 5-HT2B receptor agonist, 5-HT. The intracellular calcium change was measured by FlexStation III. The relative 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-HT2B in CHO-K1 cells was 0.45 nM. The S/B of 5-HT on 5-HT2B in CHO-K1 cells was 6.

Notes:

1. EC₅₀ value is calculated with four parameter logistic equation:

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

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) = Top/Bottom

IV. 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.

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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

V. REFERENCES

1. Hoyer D (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev.* 1994 Jun;46(2):157-203.
2. Nebigil C G (2000) Serotonin 2B receptor is required for heart development. *Proc Natl Acad Sci.* 2000 Aug 15;97(17):9508-13
3. Nebigil C G (2000) 5-hydroxytryptamine 2B receptor regulates cell-cycle progression: cross-talk with tyrosine kinase pathways. *Proc Natl Acad Sci.* 2000 Aug 15;97(17):9508-13
4. Ellis ES(1995)Mediation by 5-hydroxytryptamine2B receptors of endothelium-dependent relaxation in rat jugular vein. *Br J Pharmacol.* 1995 Jan;114(2):400-4

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