Human Recombinant 5-HT2A Serotonin Receptor Stable Cell Line

Cat. No. M00251 **Version 05232014**

I. INTRODUCTION

Catalog Number: M00251

Cell Line Name: CHO-K1/5-HT2A

Gene Synonyms: 5-HT2A;

Expressed Gene: Genbank Accession Number NM_000621; no expressed tags

Host Cell: CHO-K1

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

Stability: 16 passages

Application: Functional assay for 5-HT2A 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, 400 μg/ml G418

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-HT2A which couples to Gq/11 is expressed throughout the central nervous system in the neocortex and olfactory tubercle. 5-HT2A receptor agonists may have important clinical value in the treatment of various disorders, such as depression, anxiety, bipolar disorder, and schizophrenia. GenScript's cloned human 5-HT2A–expressing cell line is generated in the CHO-K1 host.

§: 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-HT2A and CHO-K1 cells

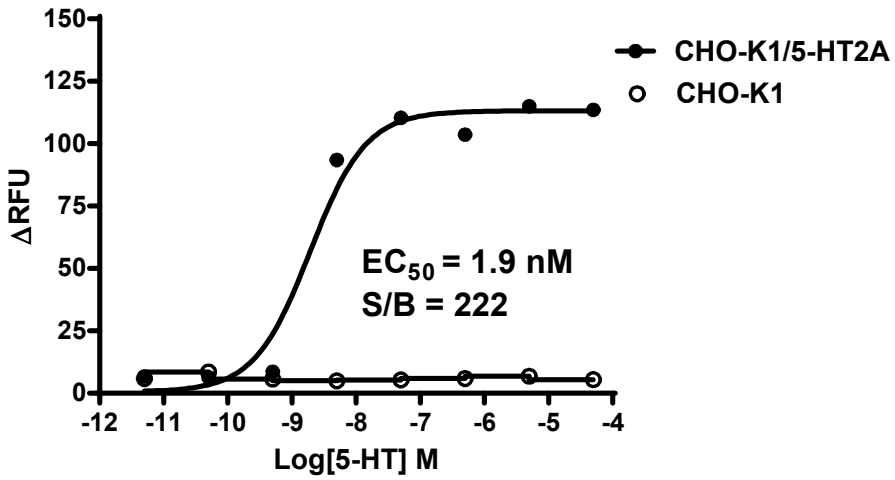

Figure 1. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/5-HT2A and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a 5-HT2A receptor agonist, 5-HT. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of 5-HT (Mean ± SD, n = 2). The EC_{50} of 5-HT on 5-HT2A in CHO-K1 cells was 1.9 nM. The S/B of 5-HT on 5-HT2A in CHO-K1 cells was 222.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*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.
6. Grow the cells in incubator with 37°C, 5 %CO_2.

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 %CO_2.

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. Meyer JH (2008)Serotonin2A receptor binding potential in people with aggressive and violent behaviour. *J Psychiatry Neurosci*. 2008 Nov;33(6):499-508

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