Human Recombinant 5-HT2C Serotonin Receptor Stable Cell Line

Cat. No. M00319 **Version 05292014**

I. INTRODUCTION

Catalog Number：M00319
Cell Line Name: HEK293/5-HT2C
Gene Synonyms: 5-HT2C; 5-HTR2C;
Expressed Gene: Genbank Accession Number NM_000868; no expressed tags
Host Cell: HEK293
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for 5-HT2C receptor
Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO
Complete Growth Medium: DMEM, 10% FBS
Culture Medium: DMEM, 10% FBS, 300 mg/ml G418
Plating medium: DMEM 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). 5HT2C is expressed in the brain and spinal cord, especially the choroid plexus. 5HT2C receptor agonists may have important clinical value in the treatment of mental and eating disorders, such as depression, panic anxiety, OCD, bulimia, and obesity.

§: 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-Hydroxytryptamine (5-HT) in HEK293/5-HT2C and HEK293 cells

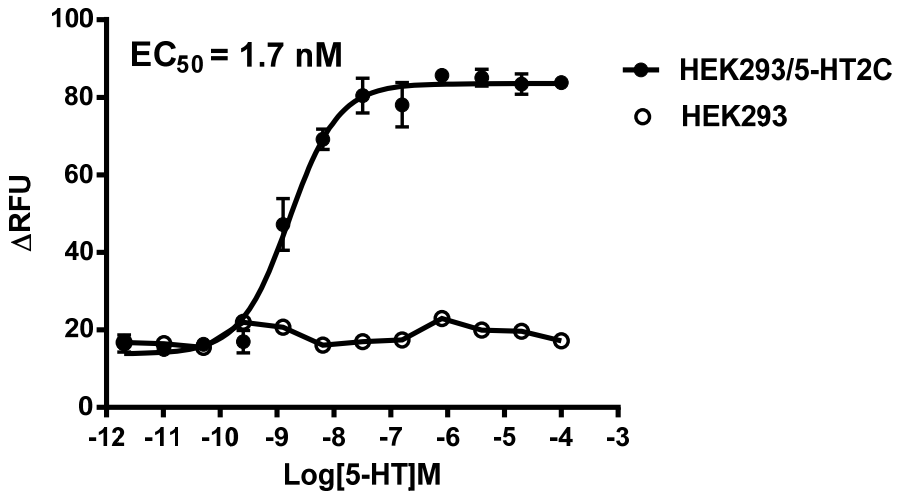

Figure 1. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in HEK293/5-HT2C and HEK293 cells. The cells were loaded with Calcium-4 prior to stimulation with a 5-HT2C receptor agonist, 5-HT. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of 5-HT (Mean ± SD, n = 2). The EC_{50} of 5-HT on 5-HT2C in HEK293 cells was 1.7 nM. The S/B of 5-HT on 5-HT2C in HEK293 cells was 6.

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. RADIOLIGAND BINDING ASSAY

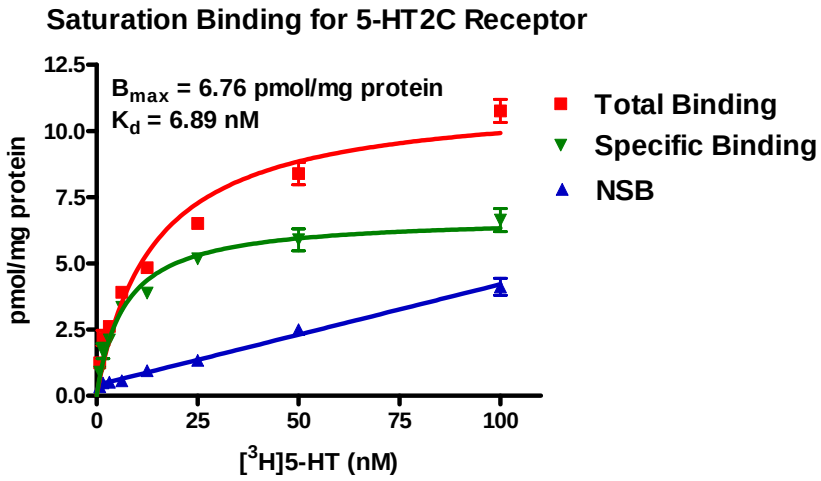

Figure 2. 5 µg of membranes prepared from HEK293 cells stably expressing 5-HT2C receptors were incubated with indicated concentrations of [^{3}H]5-Hydroxytryptamine in the absence (total binding) or presence of 1000-fold excess unlabeled Serotonin (nonspecific binding, NSB). Binding was terminated by 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.

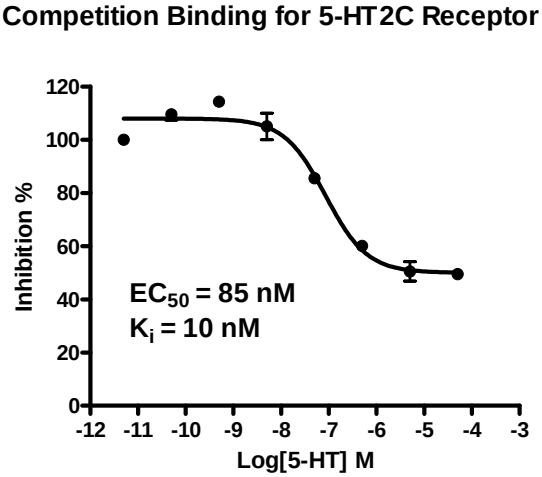

Figure 3. 5 µg of membranes prepared from HEK293 cells stably expressing 5-HT2C receptors were incubated with indicated concentrations of 5-Hydroxytryptamine (5-HT) in the presence of 50 nM[^{3}H] 5-HT. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a 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 %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

VI. 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. Werneck AL (2009) The use of an antagonist 5-HT2a/c for depression and motor function in Parkinson' disease. *Arq Neuropsiquiatr*. 2009 Jun;67(2B):407-12.
3. Mancia F (2008) Ligand sensitivity in dimeric associations of the serotonin 5HT2c receptor. *EMBO Rep.* 2008 Apr;9(4):363-9. Epub 2008 Mar 14

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