Human Recombinant 5-HT1A Serotonin Receptor Stable Cell Line

Cat. No. M00330 **Version 07302020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for 5-HT1A receptor (Calcium flux assay, cAMP assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Plating medium: Ham's F-12, 10% dialyzed FBS
Culture Medium: Ham's F-12, 10% FBS, 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen), 200 µg/ml Zeocin (Cat. #R250-01, Life Technologies)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

The 5-Hydroxytryptamine receptor 1A (5-HT1A) is G_i-coupled GPCRs expressed in the cerebral cortex, hippocampus, septum, amygdala, and raphe nucelus, 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.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE Data

Calcium mobilization assay:

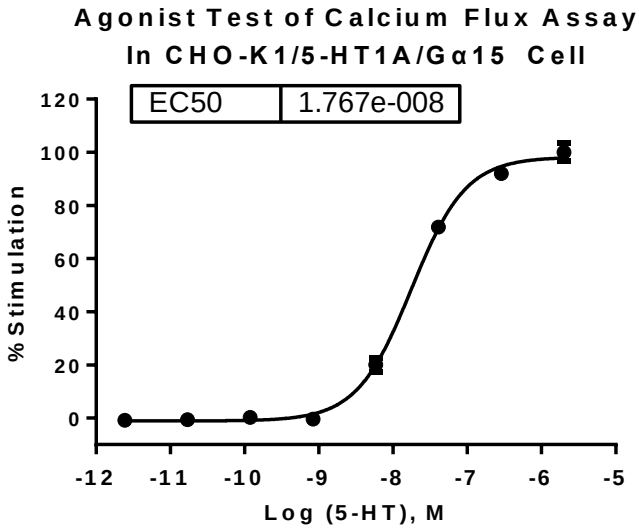

Figure 1. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/5-HT1A/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist 5-HT. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of 5-HT (Mean ± SD, n = 2). The EC_{50} of 5-HT on this cell was 17.7 nM.

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.

RADIOLIGAND Binding Assay

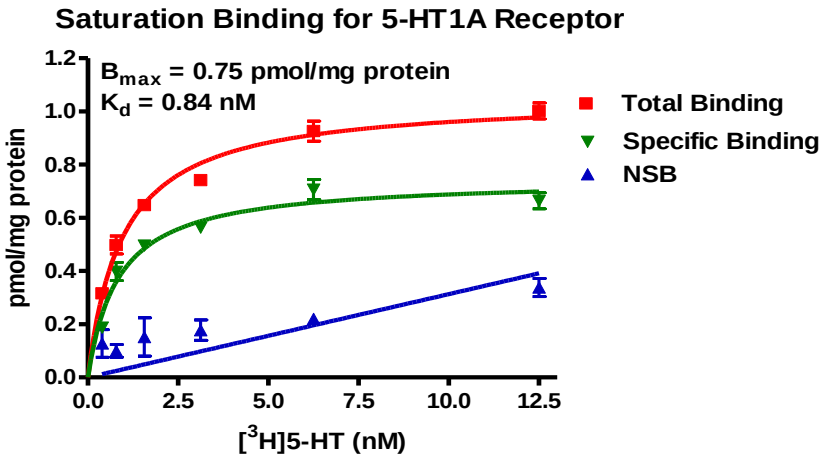

Figure 2 10 µg of membranes prepared from CHO-K1 cells stably expressing 5-HT1A receptors were incubated with indicated concentrations of [^{3}H]5-HT in the absence (total binding) or presence of 1000-fold access unlabeled Serotonin (nonspecific binding, NSB). Binding was terminated by rapid filtration. Specific binding was defined by subtracting NSB from total binding. Data were fit to one-site binding equation using a non-linear regression method.

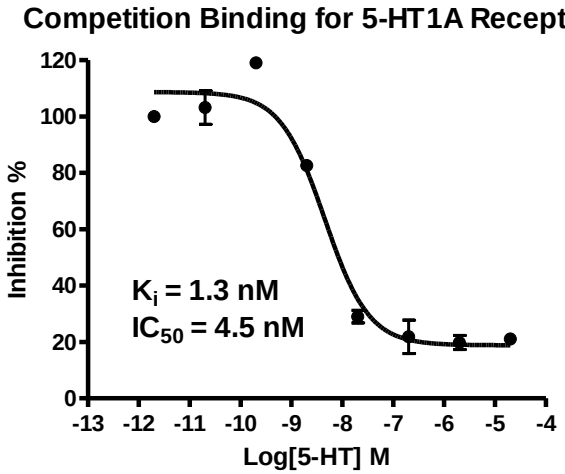

Figure 3 10 µg of membranes prepared from CHO-K1 cells stably expressing 5-HT1A receptors were incubated with indicated concentrations of 5-HT in the presence of 2 nM [^{3}H]5-HT. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.

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. Add antibiotic in the following day.

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
Medium Renewal: Every 2 to 3 days

V. 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.

GenScript USA Inc,
860 Centennial Ave.
Piscataway, NJ 08854
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188, Fax: 1-732-210-0262
Email: product@genscript.com
Web: http://www.genscript.com

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