Human Recombinant 5-HT4 Serotonin Receptor Stable Cell Line
Cat. No. M00444 **Version 07312020**

I. INTRODUCTION

Catalog Number: M00444
Cell Line Name: CHO-K1/5-HT4/Gα15
Gene Synonyms: HTR4, 5-HT4
Expressed Gene: Genbank Accession Number NM_000870; 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-HT4 receptor (Calcium flux 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
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 400 μg/ml G418 (Cat. #10131-035, Gibco), 200 μg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Plating medium: Ham's F-12K (Kaighn's)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

5-HT4 is a human serotonin G protein-coupled receptor. Several studies suggested that intact 5-HT systems are important determinants of sensitivity and/or tolerance to ethanol-induce ataxia hypothermia. Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT receptor knock-out mice. Neuropsychiatric disorders such as anxiety, depression, migraine, vasospasm and epilepsy may involve different subtypes of the 5-HT receptor.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by 5-Hydroxytryptamine (5-HT) in CHO-K1/5-HT4/ Gα15 cells

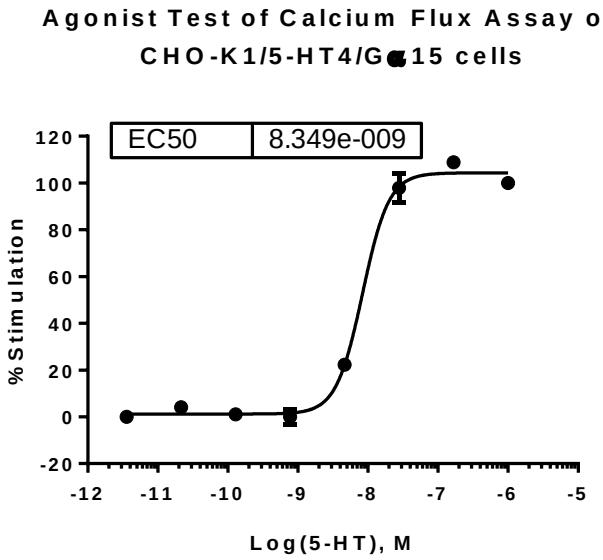

Figure 1. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/5-HT4/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with 5-HT. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of 5-HT (Mean ± SEM, n = 3). The EC_{50} of 5-HT on this cell was 8.35 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.
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. 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. Compan, V., Zhou, M., Grailhe, R., *et al.* (2004) Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT4 receptor knock-out mice.*J. Neurosci.*24(2):412-9.
2. Crabbe, J.C., Phillips, T.J., Feller, D.J., *et al*. (1996) Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors.*Nat. Genet.* 14(1):98-101.
3. Oksenberg, D., Marsters, S.A., O'Dowd, B.F., *et al.* (1992) A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT1B receptors.*Nature.* 360(6400):161-3.

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