Human Recombinant Cannabinoid Receptor 1 Stable Cell Line
Cat. No. M00299 **Version 07282020**

I. INTRODUCTION

Catalog Number: M00299
Cell Line Name: CHO-K1/CNR1/Gα15
Gene Synonyms: CNR1; CANN6; CB-R; CB1; CB1A; CB1K5; CNR
Expressed Gene: Genbank Accession Number NM_016083; no expressed tags
Host Cell: CHO-K1/Gα15
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for CNR1 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, 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 cannabinoid receptor CNR1 is Gs-coupled GPCRs expressed in primarily CNS and some peripheral neurones; particularly prevalent in basal ganglia, hippocampus, cerebellum, cerebral cortex and also presented in some non-neuronal cells and tissues, for example leukocytes and testis. A shorter human splice variant (411aa) has been identified in the brain and other tissues by reverse-transcriptase PCR but mRNA levels were less than 10-fold the levels of the longer isoform. The pharmacological characteristics of the isoforms are similar.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by CP55940 in CHO-K1/CNR1/Gα15 cells

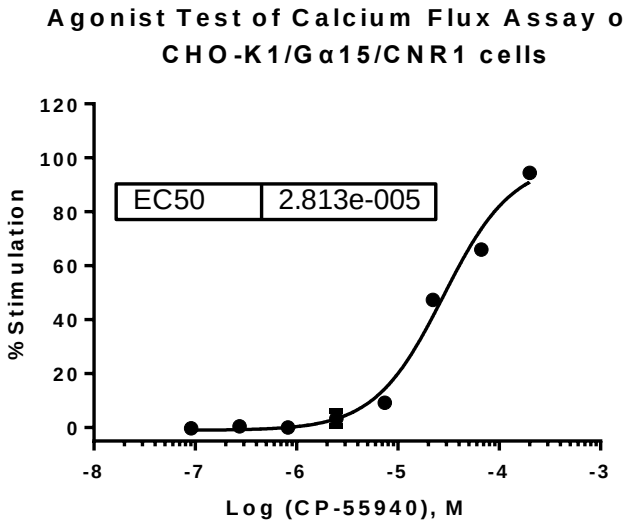

Figure 1. CP55940-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CNR1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist, CP55940. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of CP55940 (Mean ± SD, n = 2). The EC_{50} of CP55940 on this cell was 28.1 µM.

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.

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. Pertwee RG *et al.* (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther.* 74(2):129-80.
2. Herkenham M *et al.* (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A. 87(5):1932-6.
3. Howlett AC, *et al.* (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *rmacol Rev.* 54(2):161-202.
4. Shire D, *et al.* (1995) An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. *J Biol Chem.* 270(8):3726-31.

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