# Human Recombinant Cannabinoid Receptor 2 Stable Cell Line

**Technical Manual No. TM0585** **Version 10132010**



## I. Introduction

Catalog Number: M00433

Cell Line Name: CHO-K1/CB2

Gene Synonyms: CNR2; CB2; CX5

Expressed Gene: Genbank Accession Number NM_001841; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells ($3\times10^6$ per vial)

Stability: 16 passages

Application: Functional assay for CB2 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

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

## II. Background

The Cannabinoid receptor 2 (CNR2) is a $G_{i/o}$-coupled GPCR expressed in spleen, tonsils, bone marrow and peripheral blood leukocytes. There is one report that cannabinoid-induced inhibition of helper T cell activation is lost in macrophages obtained from CNR2 knockout mice. CNR2 is a potential therapeutic target in the treatment of various disease conditions, such as pain, multiple sclerosis, vascular disease, Parkinson's disease, and other central nerve system disorders.

*§: 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.*



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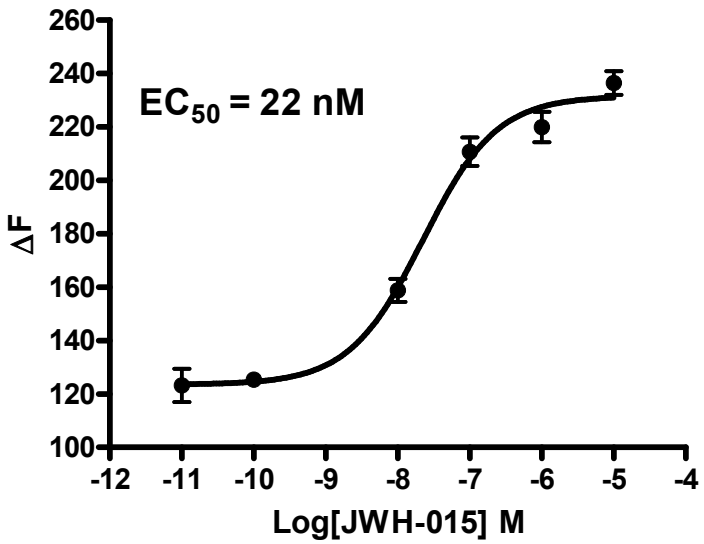
## III. Assay Development

This cell based assay is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). It is a competitive immunoassay that uses cAMP labeled with the d2 acceptor flourophore and an anti-cAMP antibody labeled with Europium Cryptate. The FRET signal decreases as cAMP concentration rises.

**Agonist Assay Protocol (According To Cisbio Document Reference: 62am4peb)**

1. Seed 5 µl CHO-K1/CB2 cells into a 384-well low volume plate, 4,000 cells per well.
2. Add 5 µl compound or JWH－015 (diluted in buffer with 2% DMSO) to each well and incubate the plate for 30 min at 23°C.
3. Add 5ul of cAMP-d2 conjugate solution to each well.
4. Add 5µl of cAMP-AB lysis buffer solution to each well.
5. Incubate the plate in the dark for one hour at 23°C.
6. Read the plate PHERAstar PLUS (BMG Labtech, Offenburg, Germany).

**Agonist Data**




**Data Analysis:**

1. Ratio = $A_{665nm}/B_{620nm}$ x 104
2. Mean Ratio = $\sum_{Ratio}/2$
3. Delta F = (Sample ratio-$Ratio_{neg}$)/$Ratio_{neg}$ x 100
4. Signal to background Ratio(S/B) = $M_{known\ agonist}$ / $M_{DMSO\ control}$
5. CV = 100 x SD/M (%)
6. Z factor = 1 - 3 x | $SD_{known\ agonist}$ +$SD_{DMSO\ control}$ | / | $Mean_{known\ agonist}$ -$Mean_{DMSO\ control}$ |



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## 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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add G418 to a concentration of 400 µg/ml the following day.

**Subculturing: Protocol**

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

## V. References

1. T Howlett AC *et al*. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev.* 2002 Jun; 54(2):161-202.
2. Buckley NE *et al*. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. *Eur J Pharmacol.* 2000 May 19; 396(2-3):141-9.

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