Human Recombinant D2 Dopamine Receptor Stable Cell Line

Technical Manual No. TM0391 **Version 10132010**

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I. Introduction

Catalog Number: M00152

Cell Line: CHO-K1/D2/Gα15

Gene Expressed: GenBank Accession Number NM_000795; no expressed tags

Gene Synonyms: DRD2, D2R, D2DR

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Applications: Functional assays for D2 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, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

Dopamine is the predominant catecholamine neurotransmitter found in mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. It also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function, and gastrointestinal motility. The dopamine receptor family consists of five members, which are classified into two groups, D1-like (D1 and D5) and D2-like (D2, D3, and D4). Dopamine receptor 2 is mainly expressed in the brain. It has splicing variants, $D2_L$ and $D2_S$. D2R receptor is implicated in a number of neurological and psychiatric conditions. Drugs acting at dopamine D2 receptors (D2R) are commonly used to alleviate symptoms for Parkinson's disease, schizophrenia, and depression.

§: 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. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Dopamine in CHO-K1/D2/Gα15 and CHO-K1/Gα15 cells

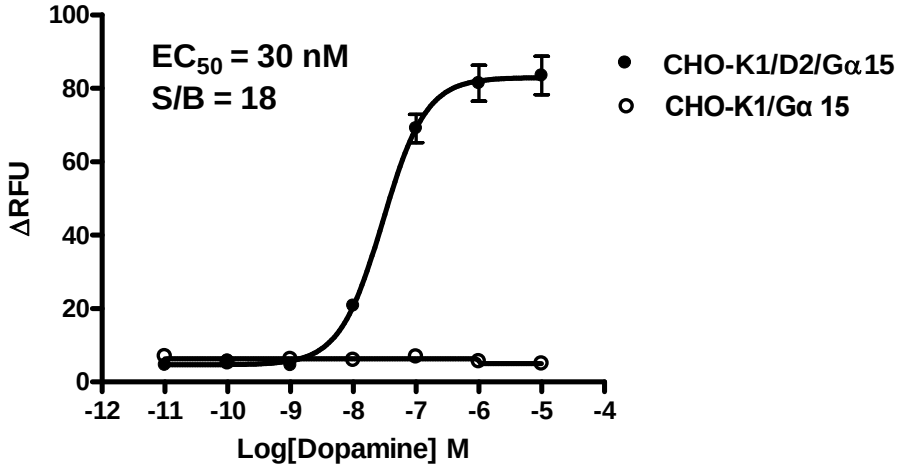

Figure 1. Dopamine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/D2/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a D2 receptor agonist, Dopamine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Dopamine (Mean ± SD, n = 2). The EC_{50} of Dopamine on D2 co-expressing with Gα15 in CHO-K1 cells was 30 nM. The S/B of Dopamine on D2 in CHO-K1 cells was 18.

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 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 Hygromycin B and Zeocin to concentrations of 100 μg/ml and 200 μg/ml respectively the following day.

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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. Stormann,T.M. (1990) Molecular cloning and expression of a dopamine D2 receptor from human retina. *Mol. Pharmacol.* 37 (1), 1-6
2. Missale C, (1998) Dopamine receptors: from structure to function. *Physiol Rev.* 78(1):189-225.

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