

Human Recombinant D5 Dopamine Receptor Stable Cell Line

Technical Manual No. TM0530

Version 10132010

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I. Introduction

Catalog Number: M00322

Cell Line: CHO-K1/D5/Gα15

Gene Expressed: GenBank Accession Number NM_000798; no expressed tags

Gene Synonyms: DRD5; DBDR; DRD1B; DRD1L2; MGC10601

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10⁶ per vial)

Stability: 16 passages

Applications: Functional assays for D5 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). D5 receptor has a 10-fold higher affinity for dopamine than the D1 receptor.

§: 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.

III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Dopamine in CHO-K1/D5/Gα15 and CHO-K1/Gα15 cells

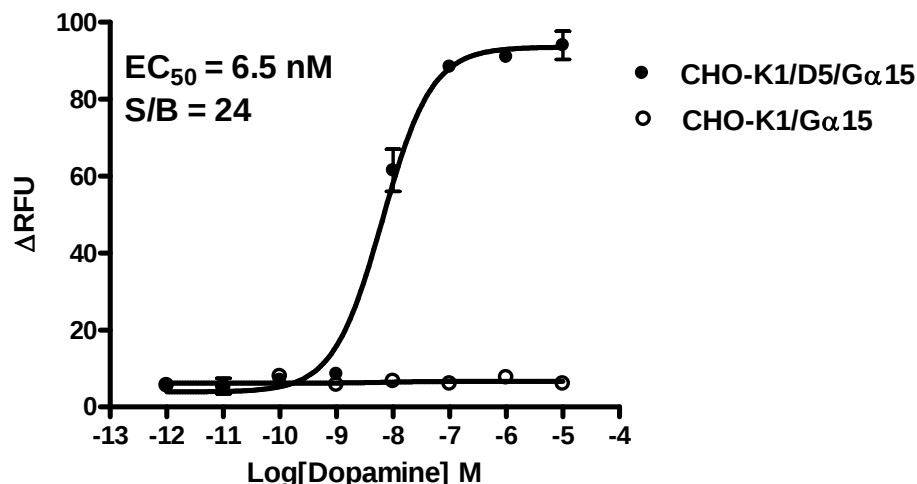


Figure 1. Dopamine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/D5/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a D5 receptor agonist, Dopamine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Dopamine (Mean ± SD, n = 2). The EC₅₀ of Dopamine on D5 co-expressing with Gα15 in CHO-K1 cells was 6.5 nM. The S/B of Dopamine on D5 co-expressing with Gα15 in CHO-K1 cells was 24.

Notes:

1. EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$$

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.

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. Bergson, C, *et al.* (1995) Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J Neurosci.* 12:7821-36
2. Dearry, A, *et al.* (1990) Molecular cloning and expression of the gene for a human D1 dopamine receptor. *Nature* 347:72-76
3. Missale C, (1998) Dopamine receptors: from structure to function. *Physiol Rev.* 78(1):189-225.

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