

Human Recombinant D1 Dopamine Receptor Stable Cell Line

Cat. No. M00247

Version 05262014

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I. INTRODUCTION

Catalog Number: M00247

Cell Line Name: CHO-K1/D1

Gene Synonyms: DRD1; DADR; DRD1A

Expressed Gene: Genbank Accession Number NM_000794; no expressed tags

Host Cell: CHO-K1

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

Stability: 16 passages

Application: Functional assay for D1 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 μ g/ml Zeocin

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).

§: 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.

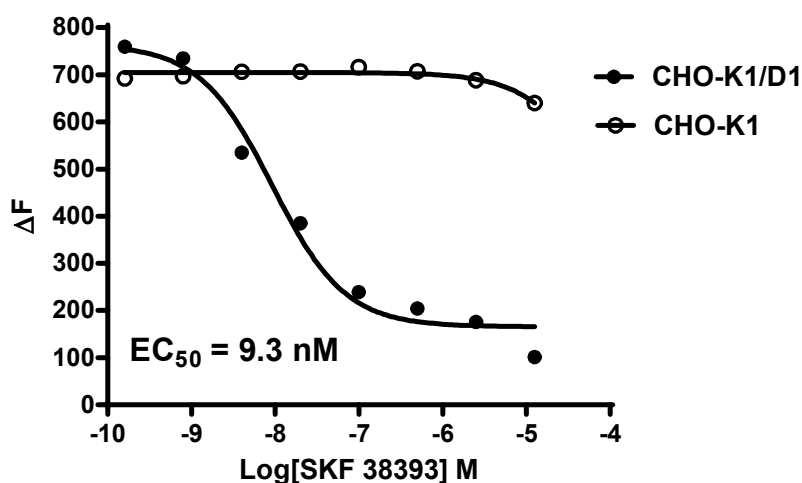
III. ASSAY DEVELOPMENT

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 fluorophore and an anti-cAMP monoclonal AB 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/D1 cells into a 384-well low volume plate, 4,000 cells per well.
2. Add 5 µl compound or SKF 38393 (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 = $A_{665nm}/B_{620nm} \times 104$
2. Mean Ratio = $\sum \text{Ratio} / 2$
3. Delta F = $(\text{Sample ratio} - \text{Ratio}_{neg}) / \text{Ratio}_{neg} \times 100$
4. Signal to background Ratio(S/B) = $M_{\text{known agonist}} / M_{\text{DMSO control}}$
5. CV = $100 \times \text{SD} / M$ (%)
6. Z factor = $1 - 3 \times \left(\frac{\text{SD}_{\text{known agonist}} + \text{SD}_{\text{DMSO control}}}{\text{Mean}_{\text{known agonist}} - \text{Mean}_{\text{DMSO control}}} \right)$

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 %CO₂.
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 %CO₂.

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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