

Human Recombinant D2S Dopamine Receptor Stable Cell Line**Cat. No. M00274****Version 05262014**

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I. INTRODUCTION

Catalog Number: M00274

Cell Line Name: CHO-K1/D2S/Gα15

Expressed Gene: GenBank Accession Number NM_016574; no expressed tags

Host Cell: CHO-K1

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

Stability: 16 passages

Applications: Functional assays for D2s 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

The short form of D2 (D2S) and the long form (D2L) are two isoforms that contribute differentially to dopamine signaling in both prefrontal cortex and striatum. The D2 dopamine receptor, short form (D2s) has been shown to stimulate phospholipase D (PLD) activity independent of the activation of phospholipase C (PLC) activity in GH4 derived cells stably transfected with the D2s receptor. Agonist activation of D2s has been shown to mediate the inhibition of growth in the same cell line.

§: 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/ D2S/Gα15 and CHO-K1 cells

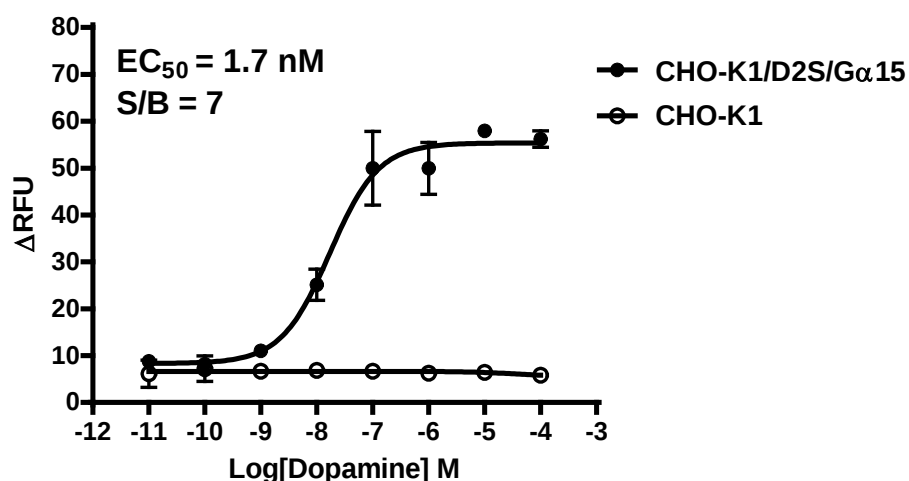


Figure 1. Dopamine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/D2S/Gα15 and CHO-K1. The cells were loaded with Calcium-4 prior to stimulation with a D2 receptor agonist, Dopamine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Dopamine (Mean ± SD, n = 2). The EC₅₀ of Dopamine on D2 co-expressing with Gα15 in CHO-K1 cells was 1.7 nM. The S/B of Dopamine on D2 co-expressing with Gα15 in CHO-K1 cells was 7.

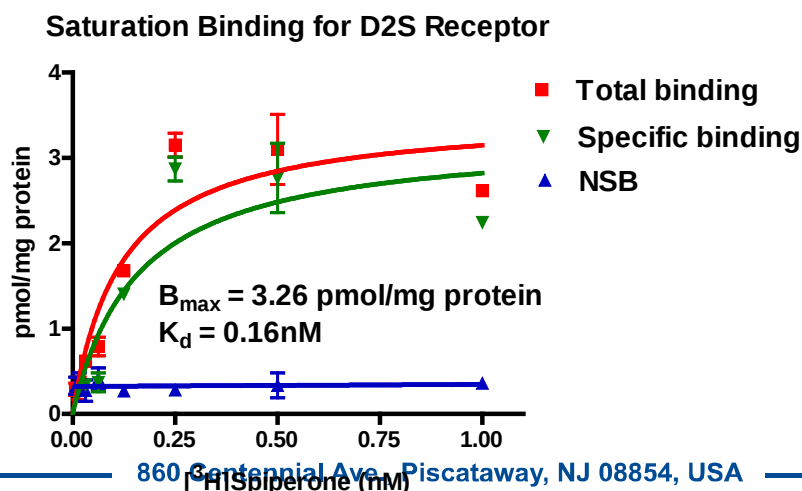
Notes:

- 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.
- Signal to background Ratio (S/B) = Top/Bottom

Radioligand Binding Assay



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Figure 2. 6 µg of membranes prepared from CHO-K1 cells stably expressing D2S receptors were incubated with indicated concentrations of [³H]Spiperone in the absence (total binding) or presence of 1000-fold excess unlabeled Haloperidol (nonspecific binding, NSB). Binding was terminated by rapid filtration. Specific binding was defined by subtracting NSB from total binding. Data were fit to one-site binding equation using a non-linear regression method.

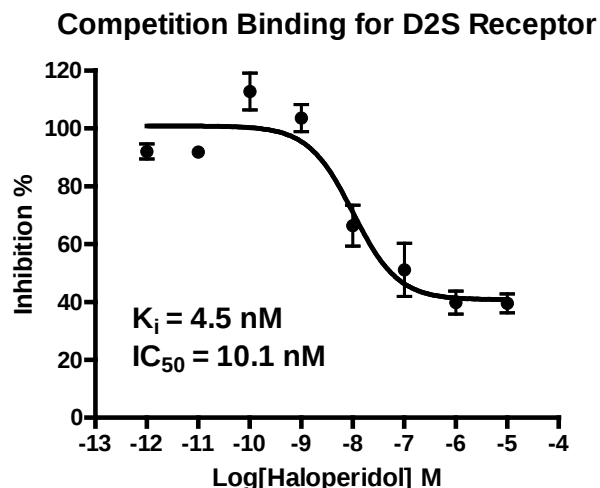


Figure 3. 6 µg of membranes prepared from CHO-K1 cells stably expressing D2S receptors were incubated with indicated concentrations of Haloperidol in the presence of 1 nM [³H]Spiperone. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.

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.

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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. Senogles SE. (2003) D2s dopamine receptor mediates phospholipase D and antiproliferation. *Mol Cell Endocrinol.* 2003 Nov 14;209(1-2):61-9

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