**Human Recombinant D2S Dopamine Receptor Stable Cell Line**
**Cat. No. M00274** **Version 07282020**



## 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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>$1\times10^6$ per vial)
Stability: More than 16 passages
Applications: Functional assays for D2s receptor (calcium flux assay, radioligand binding assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco),10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 200 μg/ml Zeocin (Cat. #R250-01, Life Technologies), 100 μg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

## 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.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

## III. REPRESENTATIVE DATA

**Concentration-dependent stimulation of intracellular calcium mobilization by Dopamine in CHO-K1/ D2S/Gα15 and CHO-K1 cells**

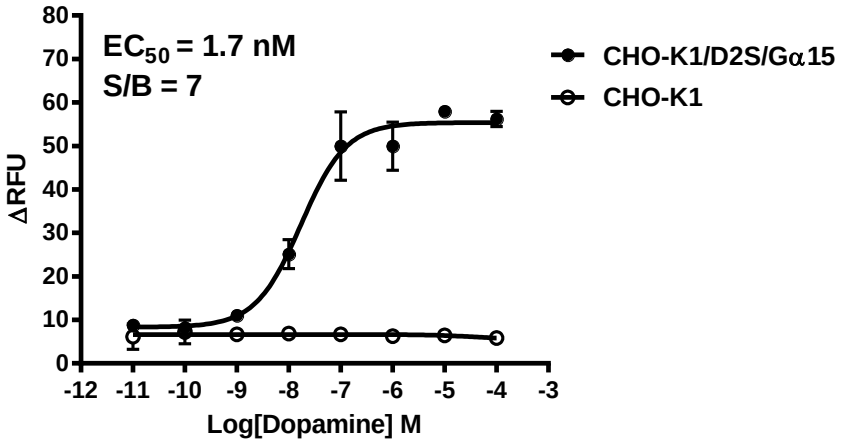


**Figure 1.** Dopamine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/D2S/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with the agonist dopamine. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (10-fold dilution) of dopamine (Mean ± SD, n = 2). The $EC_{50}$ of dopamine on this cell was 1.7 nM.

**Notes:**

1. $EC_{50}$ value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((Log$EC_{50}$-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

**Radioligand Binding Assay**

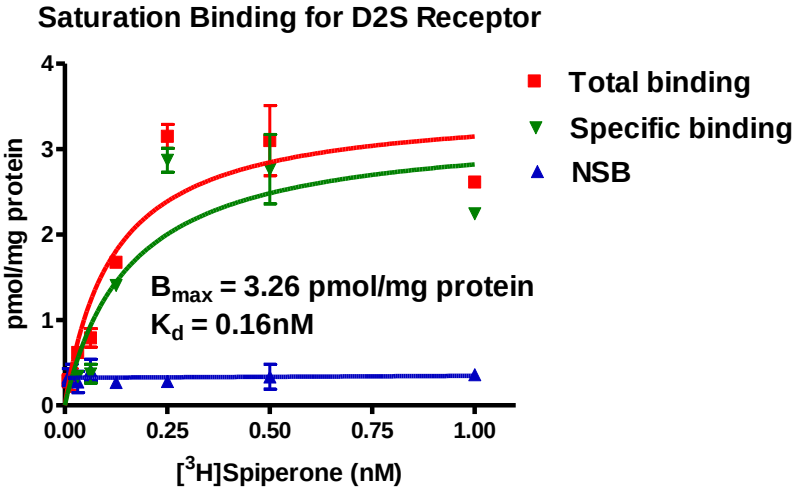


**Figure 2.** 6 µg of membranes prepared from CHO-K1 cells stably expressing D2S receptors were

incubated with indicated concentrations of [$^3$H]Spiperone in the absence (total binding) or presence of 1000-fold access 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.

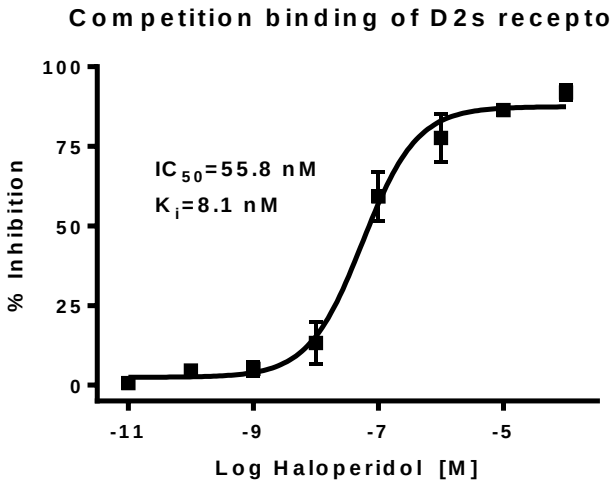


**Figure 3.** 6 µg of membranes prepared from CHO-K1 cells stably expressing D2S receptors were incubated with indicated concentrations of Haloperidol in the presence of 1 nM [$^3$H]Spiperone. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear 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 %$CO_2$.
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 %$CO_2$.

Subcultivation Ratio: 1:3 to 1:8
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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