Human Recombinant D2S Dopamine Receptor Stable Cell Line

Technical Manual No. TM0435

I. Introduction

Catalog Number: M00274

Cell Line Name: CHO-K1/D2S/Ga15

Expressed Gene: GenBank Accession Number NM_016574; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10⁶ 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.
III. Representative Data

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

![Graph showing concentration-dependent stimulation of intracellular calcium mobilization](image)

**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 D2S 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 D2S co-expressing with Gα15 in CHO-K1 cells was 1.7 nM. The S/B of Dopamine on D2S co-expressing with Gα15 in CHO-K1 cells was 7.

Notes:

1. EC\(_{50}\) value is calculated with four parameter logistic equation:
   \[ Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1+10^{(\text{LogEC}_{50}\cdot X)\cdot \text{HillSlope}})} \]
   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
Radioligand Binding Assay

**Saturation Binding for D2S Receptor**

- **Total binding**
- **Specific binding**
- **NSB**

![Graph showing saturation binding for D2S receptor]

- $B_{\text{max}} = 3.26 \text{ pmol/mg protein}$
- $K_d = 0.16 \text{nM}$

**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 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.

**Competition Binding for D2S Receptor**

![Graph showing competition binding for D2S receptor]

- $K_i = 4.5 \text{ nM}$
- $IC_{50} = 10.1 \text{ nM}$

**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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 2 ml of the cell suspension per well 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 5 min, 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

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