Human Dopamine D2 Receptor Cell Line

Technical Manual No. TM0307 **Version 20080820**

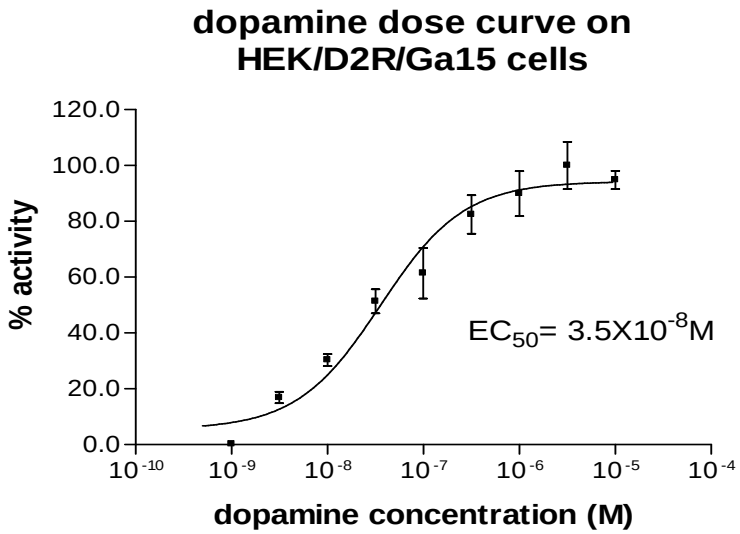
I. Introduction

The diverse physiological effects of dopamine are mediated through cell surface G protein-coupled dopamine receptors. To date, five subtypes of dopamine receptors have been cloned: D1, D2, D3, D4, and D5. The dopamine D2 receptor couples to inhibitory G proteins (Gi and Go) and inhibits adenylyl cyclase with subsequent decrease of cAMP[1]. In addition, D2 receptors can also increase outward potassium currents, leading to cell hyperpolarization[2]. D2 receptors have been reported as expressed in the brain, heart, blood vessels, adrenal glands, kidneys, and sympathetic ganglia[3,4]. The D2 receptors play important role in the regulation of movement, cognitive and emotional responses, and vasodilation in the kidney. Hence, D2R-agonists may constitute one possible avenue of treatment for Parkinson's disease and hypertension. This manual describes establishment of a cell line and a protocol of pharmacologically validated human gonadotropin-releasing hormone receptor (Genebank Accession Number: NM_000795).

II. Cell Line Information

- Catalog Number：M00129
- Cell Line Name: HEK/D2R/Gα15
- Description:
  The D2R reporter cell line is created by transfection of pMT8-D2R in a parental cell line, HEK293/Gα15. The transfected cells are stably selected by 3 μg/ml puromycin. Single-cell clones with high D2R inducibility and low calcium current background are isolated using ring cloning and serial dilution. The clones with largest dynamic range in calcium current are chosen for pharmacological and stability studies.
- Cell Line Name: HEK/D2R/Gα15
- Function: Cell based, functional assay for D2R receptor
- Quantity: 2 vial (2×10^6) frozen cells
- Passage Number Shipped: 2
- Host Cell: HEK293
- Cell Phenotype: Adherent/epithelial
- Mycoplasma: Negative
- Recommended Storage: Liquid nitrogen upon delivery
- Propagation Medium: DMEM, 10% FBS, 3 μg/ml puromycin

III. Dopamine Dose Response of HEK/D2R/Gα15 Cell Line and Assay Procedure



1. Cells are seeded at a density of 3×10^4 to 5×10^4 cells/well in a 96-well black plate 12-24 hours before running the assay.
2. The day of the assay, cells should be 100% confluent. Remove cell plates from the incubator. Do not remove the supernatant. Add an equal volume of Fluo3 Loading Buffer to each well (100 µl per well for 96-well plates, 25 µl per well for 384 well plates).
3. Incubate the plate at 37°C in the dark for one hour.
4. Prepare agonist addition plates in advance of assay. To a 96-well plate, add 10X working concentration of agonist compound in calcium assay solution , and add 25 µl/well to cell plate.
5. Read with FlexStation or FLIPR using the specified settings and save data. The assay should be completed within five minutes of addition, but we recommend collecting data for a minimum of six minutes during assay development. Usually 90 seconds are enough.

Instrument Optical Parameters	
Excitation wavelength (nm)	485
Emission wavelength (nm)	525
Emission cut-off (nm)	515

IV. References

1. Sibley, DR & Monsma, FJ, Jr. Molecular biology of dopamine receptors. Trends Pharmacol Sci, 1992, 13:61-9.
2. Greif, G. J., Lin, Y. J., Liu, J. C. & Freedman, J. E. Dopamine-modulated potassium channels on rat striatal neurons: specific activation and cellular expression. J Neurosci, 1995, 15:4533-44.
3. Bouthenet, M. L. *et al*. Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res, 1991, 564:203-19.
4. Gao, D. Q., Canessa, L. M., Mouradian, M. M. & Jose, P. A. Expression of the D2 subfamily of dopamine receptor genes in kidney. Am J Physiol, 1994, 266:F646-50.

V. Appendix

Cell Culture Conditions

Complete Culture Medium:

DMEM: 90%, FBS: 10%, L-glutamine 2.0 mM, Amp 100 µg/ml, Strep 100 µg/ml, puromycin 1.5 µg/ml

Freezing Medium:

20% of fetal bovine serum and 10% dimethyl sulphoxide (DMSO)

Thawing Cells:

1. Quickly thaw frozen cells in a 37°C water bath, agitating continuously.
2. Using a 1 ml pipette, slowly pipet the cells up and down five times and add, drop by drop, to a 15 ml centrifuge tube containing 5 ml of fresh prewarmed complete DMEM medium. Then centrifuge at 1000 rpm for five minutes.
3. Discard the supernatant medium and resuspend the cell pellet in 5 ml of fresh prewarmed complete DMEM medium. Transfer cells to a T25 flask and incubate at 37°C with 5% CO_2 until the cells reach >90% confluence. The recovery rate for frozen cells is usually 90% or above.

Subculturing:

When the cells reach confluence, they need split. This cell line is normally split twice weekly at 1:8 to 1:15 dilutions.

1. Carefully aspirate all the media. Gently rinse the cell layer with appropriate amount of 0.2% trypsin-EDTA, and aspirate it off.
2. Wait for about 1-3 minutes. Then dislodge the cells by gently tapping the sides of flask or dish.
3. Resuspend cells with appropriate amount of complete DMEM medium, and split cells as desired.

Changing Medium:

This is normally done every other day.

1. Gently aspirate off medium.
2. Transfer fresh warm complete DMEM medium (37°C) into a flask (5 ml for T25 and 10 ml for T75).

Freezing Cells:

1. Repeat steps 1-3 of subculturing section.
2. Centrifuge down the cells at 1,000 rpm for five minutes.
3. Aspirate off the supernatant and resuspend the cells in fresh freezing medium at a density of 2-3 × 10^6 cells/ml. Add 1 ml cells per cryogenic vial.
4. Put the cryogenic vial of cells into cryo freezing container. Then transfer the container into a -80°C environment and leave it there overnight.
5. Transfer cryogenic vial into liquid nitrogen (-196°C).

Reagents & Consumables:

1. DMEM: Dulbecco's Modified Eagle Medium powder, high glucose (Gibco BRL, Cat #12100-046)
2. FBS: Fetal Bovine Serum (Hyclone, Cat #CH30160.03)
3. L-Glutamine: 200 mM (Gibco BRL, Cat # 25030-081)
4. Ampicillin: 50 mg/ml (Sigma A-9518)
5. Streptomycin Sulfate: 50mg/ml (Gibco BRL, Cat # 11860-038)
6. Puromycin (Sigma, Cat #J593)
7. Trypsin: 1:250 rom Bovine Pancreas (Gibco BRL, Cat # 27250016)

8. DMSO: dimethyl sulphoxide, for molecular biology (Sigma, Cat #D8418)
9. Hepes: Sigma Cat #H-3375
10. Dopamine: Sigma Cat #H8502
11. HBSS: (GibcoBRL Cat #14025-092
12. D-Glucose: (GibcoBRL Cat 15023-013)
13. CaCl2: (Sigma Cat #C3306)
14. Fluo3/AM: (Molecular Probe. Cat #F14242)
15. Dopamine: (Sigma Cat #H8502)
16. probenecid: (Sigma Cat #P 8761)
17. polyethylene glycol: (Sigma Cat #p3265)
18. 96 Well Plate: Costar, Cat# 3603, Blackwall/clear bottom, Polystyrene, sterilized.

Media and Solutions:

1. PBS (for preparation of 500 ml)
   1) KCl: 0.1 g
   2) KH_2PO_4: 0.1 g
   3) NaCl: 4.0 g
   4) $Na_2HPO_4.12H_2O$: 1.4425 g

Dissolve the above components in double-distilled water (ddH_2O) and adjust pH to 7.4 with 0.1 N NaOH. Add ddH_2O to the final volume of 500 ml. Autoclave and store at 4°C.

2. Trypsin-EDTA (for preparation of 100 ml)
   1) Trypsin: 0.25 g
   2) 2%EDTA: 2 ml
   3) PBS: 98 ml

Dissolve trypsin in 2%EDTA and PBS completely; sterilize the solution by passing through a 0.20 µm membrane filter; store at 4°C.

3. Culture medium (for preparation of 1 L)
   1) Measure out 950 ml distilled water to dissolve the media components with gentle stirring until the solution becomes clear.
   2) Add $NaHCO_3$ 3.7 g for high glucose DMEM
   3) Adjust pH of medium to 0.2-0.3 below the desired final working pH (using 1 N NaOH or 1 N HCl is recommended). Add slowly with stirring.
   4) Dilute to 1 liter with ddH_2O.
   5) Sterilize the medium immediately using the method of membrane filtration. Store at 4°C

4. Ampicillin/Streptomycin 50 mg/ml

Dissolve 1 g Ampicillin or Streptomycin in 20 ml ddH2O and sterilize the solution by membrane filtration using 0.20 µm filter. Aliquot and store at 4°C for short-term conservation and -20°C for long term conservation.

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Catalog Number：M00129

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