

Human Dopamine D5 Receptor Cell Line

Technical Manual No. TM0304

Version 20080820



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I. Introduction

Dopamine has been shown to be an important regulator of blood pressure, sodium balance, and renal and adrenal function through an independent peripheral dopaminergic system. The effects of dopamine are exerted by two subfamilies of cell surface receptors: D1-like and D2-like receptors, which belong to the rhodopsin-like family (family A) of G protein-coupled receptors. D1-like receptors (D1 and D5) are coupled to the stimulatory G proteins GaS and Golf and stimulate adenylyl cyclases, whereas D2-like receptors (D2, D3, D4) are coupled to the inhibitory G protein Gai and Go and inhibit adenylyl cyclases. The recently discovered D5R has received much attention from both domestic and foreign enterprises for its agonist activities and potential in the treatment of hypertension. This manual describes establishment of a cell line and a protocol of pharmacologically validated human gonadotropin-releasing hormone receptor (Genebank Accession Number: BC009748).

II. Cell Line Information

- Catalog Number: M00130
- Cell Line Name: HEK/D5R/CRE/ β la
- Description:

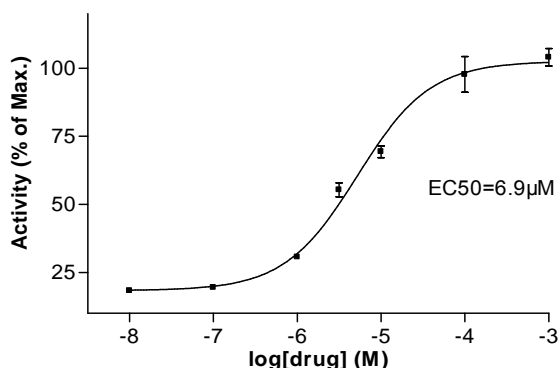
The human D5R is amplified by PCR using a high fidelity enzyme and subcloned into the pcDNA3.1/G418(+) mammalian expression vector. The full-length ORF has been confirmed by sequencing. The D5R cell line is created by cotransfection of pcDNA3.1/D5R/G418(+) with pcDNA3.1/hygromycin(+) in a parental cell line, HEK293/CRE/ β -lactamase. The transfected cells are stably selected by 100 μ g/ml hygromycin. Single cell clones with high D5R receptor inducibility and low β -lactamase background are isolated using ring cloning. The clones with largest dynamic ranges in β -lactamase are chosen for pharmacological and stability studies.
- Function: Cell based, functional assay for D5R
- Quantity: 2 vial (2×10^6 /vial) frozen cells
- Host Cell: HEK293
- Cell Phenotype: Adherent/epithelial
- Recommended Storage: Liquid nitrogen, upon delivery
- Propagation Medium: DMEM, 10% FBS, 100 μ g/ml hygromycin.

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III. Dopamine Dose Response Curve of HEK293/D5R/CRE/ β la Cell Line and Assay Procedure

Dopamine Dose Response Curve of HEK293/D5R/CRE/ β la Cell Line



1. Seed 25,000 cells per well in Growth Medium (100 μ l per well) into 96-well tissue culture treated black-wall, clear-bottom plates (Costar #3603) after trypsinization. Prepare some wells with medium alone (no cells) to use for determining plate background.
2. Culture cells in 5% CO₂ at 37°C. Allow cells to reach \approx 90% confluence.
3. 12-24 hours before the assay, replace Growth Medium with 100 μ l/well serum-free DMEM. Be careful not to disturb the cells.
4. Prepare ligand solution in serum-free DMEM (10X).
5. Add 10 μ l of 10X ligand solution to wells for stimulation and 10 μ l of serum-free DMEM per well for non-stimulated control.
6. Incubate cells in 5% CO₂ at 37°C for 5-6 hours.
7. Load cells with 2 μ M CCF4/AM as described in CCF4 Loading Protocol.
8. Incubate the plate at room temperature for 60-120 minutes without shaking.
9. Read with Analyst HT plate reader or similar fluorescence plate reader.

IV. References

1. Hollon, T. R. *et al.* Mice lacking D5 dopamine receptors have increased sympathetic tone and are hypertensive. *J Neurosci*, 2002, 22:10801-10.
2. Jose, P. A., Eisner, G. M. & Felder, R. A. Dopamine receptor-coupling defect in hypertension. *Curr Hypertens Rep*, 2002, 4:237-44.
3. Perrichot, R. *et al.* Locally formed dopamine modulates renal Na-Pi co-transport through DA1 and DA2 receptors. *Biochem J*, 1995, 312 (Pt2):433-7.

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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, hygromycin 100 µg/ml

Serum-free DMEM:

Same as above but with no FBS
0.1% BSA

Freezing Medium:

Complete culture medium plus 20% FBS and 10% 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 1,000 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% CO₂ 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 $2-3 \times 10^6$ cells/ml. Add 1 ml cells per cryogenic vial.
4. Put the cryogenic vial of cells into cryo freezing container. Then transfer the container to a -80°C environment and leave it there overnight.
5. Transfer cryogenic vial into liquid nitrogen (-196°C).

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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: 50 mg/ml (Gibco BRL, Cat # 11860-038)
6. Hygromycin B in PBS, 50 mg/ml (Invitrogen, Cat #10687-010)
7. Trypsin: 1:250 rom Bovine Pancreas (Gibco BRL, Cat # 27250016)
8. DMSO: dimethyl sulphoxide (Sigma, Cat #D8418)
9. Hepes: Sigma Cat #H-3375
10. CCF4: (Invitrogen, Cat #K1096)
11. Dopamine: (Sigma Cat #H8502)
12. Venor[®] GeM Mycoplasma Detection Kit: Minerva Biolabs Cat #11-1050
13. 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 ₂ PO ₄ : | 0.1 g |
| 3) NaCl: | 4.0 g |
| 4) Na ₂ HPO ₄ .12H ₂ O: | 1.4425 g |

Dissolve the above components in double-distilled water (ddH₂O) and adjust pH to 7.4 with 0.1 N NaOH. Add ddH₂O 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.

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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 ddH₂O.
- 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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