HEK293 Recombinant Promiscuous G Protein $G\alpha_{15}$ Cell Line

Technical Manual No. TM0299 **Version 11242008**

I. Introduction

The murine G-protein α-subunit $G\alpha_{15}$ belongs to the Gαq protein subfamily, and it is also a promiscuous G protein because $G\alpha_{15}$ can not only couple to Gq-coupled GPCRs but also to Gi-coupled GPCRs. $G\alpha_{15}$ is widely used in GPCR-related research and pharmaceutical discoveries to change Gi-coupled GPCRs into Gq-coupled GPCRs, which are more easily detected by Ca^{2+} mobilization assays because when $G\alpha_{15}$ is coupled by GPCRs, it can activate phospholipase C, increasing inositol trisphosphate, releasing Ca^{2+} from intracellular stores.

This manual describes establishment of a cell line and a protocol of pharmacologically validated for Gi-coupled GPCRs researches or Gi-coupled-GPCRs targeted high-throughput screening assay development. The cell line is stable through at least passage 15 and demonstrates a good dynamic range in fluorescent Ca^{2+} mobilization assays.

II. Cell Line Information

- Catalog number: M00142
- Cell Line Name: HEK293/$G\alpha_{15}$
- Description:
  The Gα15 cDNA is amplified by PCR using a high fidelity enzyme and subcloned into the pIRES mammalian expression vector. The full-length ORF has been confirmed by sequencing. The $G\alpha_{15}$ cell line is created by transfection of pIRES/Gα15 in HEK293 cells. The transfected cells are stably selected by 250 μg/ml hygromycin. Single cell clones with high $G\alpha_{15}$ inducibility and low background are isolated using ring cloning and serial dilution. The clones with largest dynamic range in Ca^{2+} mobilization are chosen for pharmacological and stability studies.
- Function: Cell based, functional assay for $G\alpha_{15}$
- Quantity: 1 vial (2×10^6) frozen cells
- Passage Number Shipped: 2
- Stability Tested to: 16
- Host Cell: HEK293
- Cell Phenotype: Adherent/epithelial
- Antibiotics Selection: Hygromycin
- Recommended Storage: Liquid nitrogen, upon delivery
- Propagation Medium: DMEM, 10% FBS, 100 μg/ml hygromycin, P/E
- Mycoplasma: Negative

III. Results

Because $G\alpha_{15}$ is only a G protein, we must transfect a Gi-coupled GPCR into HEK/$G\alpha_{15}$ cells to verify that HEK293/G_{15} cells truly express functional $G\alpha_{15}$ proteins.

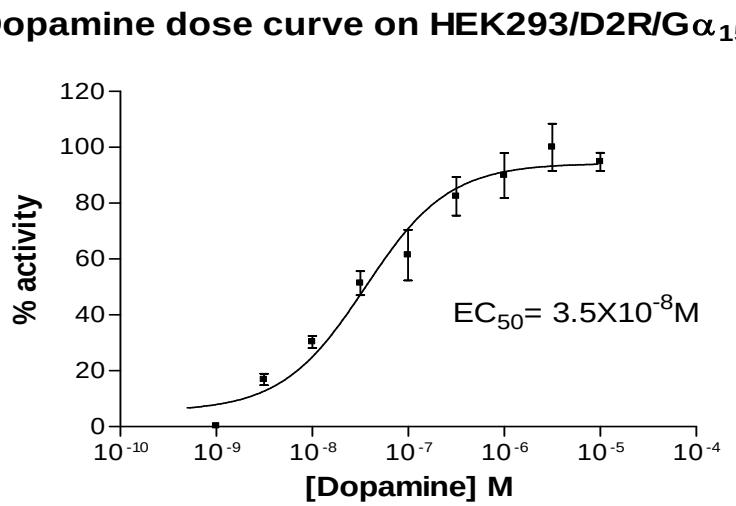


Fig. 1. Dose response of calcium currents as monitored with FlexStation plate reader upon treatment with dopamine. Assay was performed in accordance with the procedure described as below. Data represent means ± SEM for triplicate samples (left one). EC50 value for dopamine dose response was determined using GraphPad Prism 4 software (right one).

Note: The HEK293/D2R/Gα_{15} stable cell line was established based on HEK293/Gα_{15} cells, which had been frozen and thawed twice.

Assay Procedure

Fluorescent Ca^{2+}-mobilization Assay of HEK293/Gα_{15} Cells

1. Lightly tripsinize cells and seed them into 96-well plate (Metrigel coating) at 25,000 cells/well (100 µl per well) in complete culture medium.
2. Culture cells in 5% CO_2 at 37°C for 24 hours (at least overnight).
3. Discard the growth media carefully using multi-channel pipette.
4. Wash cells once carefully with 100 µl/well HBSS (containing 1 mM probenecid) using multi-channel pipette.
5. Stain cells with Fluo3/AM. Reconstitute the Fluo3/AM to a concentration of 2.0 mM for the stock solution. Dilute the Fluo3/AM of 2 mM stock to 4 µM in HBSS as the loading solution (containing1 mM probenecid, 1X red dye). Stain cells with 100 µl/well of the loading solution.
6. Incubate the plate at 37°C in the dark for one hour.
7. Prepare agonist addition plates in advance of assay. To a 96-well plate, add 5X working concentration of Dopamine in HBSS, and add 25 ul/well to cell plate.
8. Read with Flexstation using the specified settings and save data.

Note: All HBSS buffer used here containing 1 mM probenicid, including dye loading buffer, washing buffer and buffer to prepare agonist, etc. This solution is prepared fresh by diluting 200 mM probenecid stock solution (Dissolved in 200 mM NaOH) into HBSS Assay Buffer.

IV. References

1. $G\alpha_{15}$ and $G\alpha_{16}$ couple a wide variety of receptors to phospholipase C.Offermanns S, and Simon M. JBC 270(25), 15175-15180, 1995.
2. Gz signaling: emerging divergence from Gi signaling. Ho M.K.C. and Wong Y.H. Oncogene 20, 1615-1625, 2001.

V. Appendix

Cell Culture Conditions

Note: This clone often grows as clumps. It is necessary to separate cells into single cell suspensions whenever the cells are passaged or plated. MATRIGEL Matrix-coated flasks or plates are recommended for healthier cells.

Complete Culture Medium:

DMEM: 90%, FBS: 10%, L-glutamine:2.0 mM, Penicillinum:100μg /ml,Strep:100 μg/ml, hygromycin: 150 μg/ml

Freezing Medium:

Growth medium plus 45% 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% 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 the 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 to 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. Penicillin-Streptomycin,liquid (Gibco BRL, Cat # 15070-063)
5. Hygromycin B in PBS, 50 mg/ml (Invitrogen, Cat #10687-010)
6. 0.05%Trypsin-EDTA: (Gibco BRL, Cat # 25300)
7. DMSO: dimethyl sulphoxide, for molecular biology (Sigma, Cat #D8418)
8. Hepes: (Sigma Cat #H-3375)
9. Cryogenic Vial: (Corning Cat #430289)
10. Fluo-3 AM (Molecular Probes 28B2-4)
11. Probenecid: (Sigma Catalog #P 8761)
12. Dopamine: (Sigma Cat #H8502)
13. 96 Well Flat Clear Bottom White Polystyrene TC-Treated Microplates (Corning, #3610)

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)	$Na_2HPO_4.12H_2O$:	1.4 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.

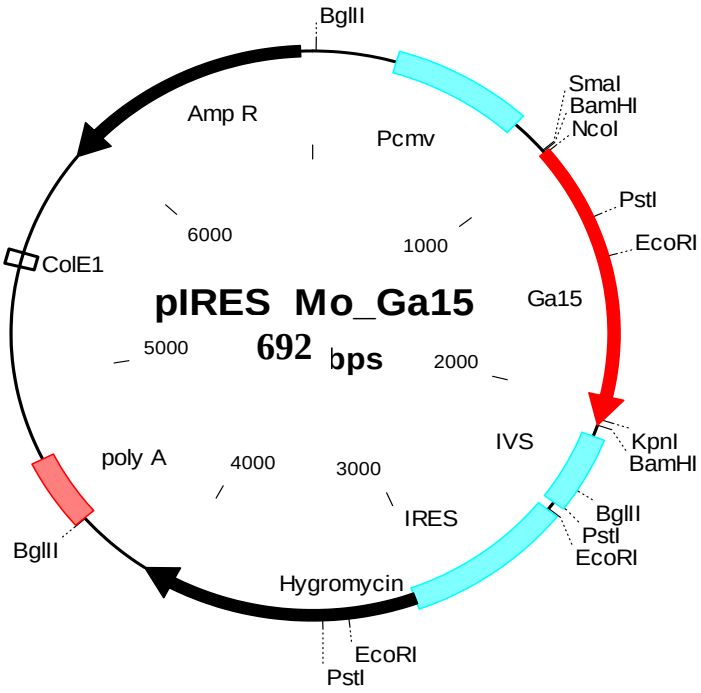
2. Culture medium (for preparation of 1 L)

1) Measure out 950 ml distilled water to dissolve the media components, stirring gently 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 while stirring.
4) Dilute to 1 liter with ddH_2O.
5) Sterilize the medium immediately using the method of membrane filtration.
   Store at 4°C.

Map of pIRES/ Gα15

Name: pIRES/mGα15
Insert gene: Murine Gα15
Length of insert: 1128bp Sequence reference: Gene Bank M80632
Vector: pIRES (Clontech) Insert site : BamH I

Plasmid Map:



Comments for pIRES/Gα15 6922 nucleotides

CMV promoter: bases 309-810
Gα15: bases 963-2090
Synthetic intron (IVS): bases 2136-2431
IRES: bases 2467-3069
Hygromycin resistance gene: bases 3067-4098
polyA signal: base 4391-4667
ColE1 origin of replication: base 5165-5784
Ampicillin resistance gene: bases 5917-6856

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Catalog Number: M00142

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