CHO-K1/CRE/Luciferase Parental Cell Line

Technical Manual No. TM0302 **Version 11242008**

I. Introduction

G-protein coupled receptors (GPCRs) represent a very large family of proteins that control many major physiological processes and are the targets of many effective drugs. GPCRs can be divided into several subfamilies according to the kind of Gα protein to which they couple. Gs-coupled GPCRs constitute a GPCR subfamily that links to Gαs proteins, which can activate adenyl cyclase and increase the intracellular concentration of cAMP. The cAMP second messenger system ultimately activates CRE and induces gene expression. Firefly luciferase is a reporter gene that is widely in the high-throughput screening for drug discovery. Here we employ both a 4xCRE element and firefly luciferase as reporters to establish parental cell lines that can be used for research involving GPCRs, particularly Gs-coupled GPCRs.

This manual describes a cell line and an assay protocols established and pharmacologically validated for the CRE-luciferase reporter system. This cell line is stable through at least passage 15 and demonstrates an approximately 30-fold dynamic range in the luciferase assay, thereby providing a good screening window. The CRE-luciferase reporter system is ready for high-throughput screening either as a primary or follow-up and can be used to identify relative GPCRs' agonists and antagonists.

II. Cell Line Information

- Catalog Number: M00145
- Cell Line Name: CHO-K1/CRE/Luc
- Description:
  The luciferase gene is subcloned from a pGL3-basic vector and inserted into a p4xCRE-blax vector and then formed into the plasmid p4xCRE-luciferase. The full-length plasmid is confirmed by sequencing. The CRE-luciferase cell line was created by transfection of p4xCRE-luciferase in HEK293. The transfected cells were stably selected by 800 μg/ml G418. Single cell clones with high CRE inducibility and low luciferase background were isolated using ring cloning and serial dilution. The clones with largest dynamic range in luciferase were chosen for pharmacological and stability studies.
- Function: Cell based, functional assay for Gs,Gi-coupled GPCRs
- Quantity: 1 vial (2×10^6) frozen cells
- Passage Number Shipped: 2
- Stability Tested to: 16
- Host Cell: CHO-K1
- Cell phenotype: Adherent/epithelial
- Antibiotics Selection: G418
- Freeze Medium: Growth medium plus 45% FBS and 10% DMSO
- Plasmid: p4xCRE-luciferase
- Recommended Storage: Liquid nitrogen upon delivery
- Propagation Medium: F12 ham's, 10% FBS, 800 μg/ml G418
- Mycoplasma: Negative

III. Results and Assay Procedure

CRE Inducibility of CHO-K1/CRE/Luc Cell Line:

	Un-stimulated Control	Stimulated (forskolin)
Background subtracted luciferase readout (RLU)	2100	16136
Fold induction	1	8

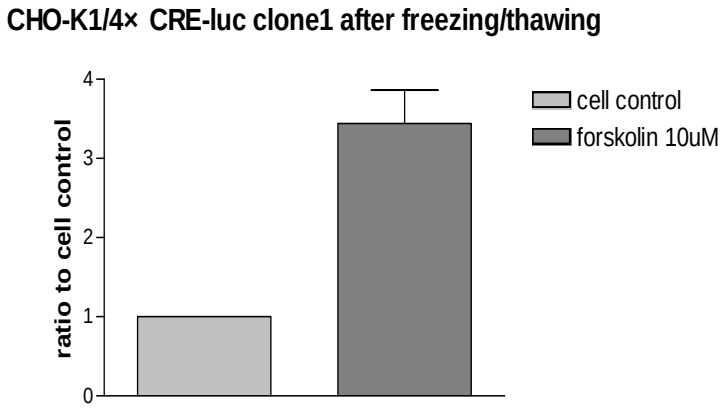


Fig. 1. **Luciferase activities were monitored with Analyst HT plate reader upon treatment with forskolin. CHO-K1/CRE/Luc cell line was treated with forskolin as indicated. Assay was performed in accordance with the procedure described above. Data represent means ± SEM for duplicate samples.**

Assay Procedure

Luciferase Assay of CHO-K1/CRE/Luc Reporter Cells:

1. Cells are lightly trypsinized and seeded into 96-well plate 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. Add 10ul 10Xforskolin reagent into each well, being careful to not dislodge attached cells. Incubate cells in 37°C/CO_2 for six hours.
4. To each well, add 100 µl of Bright-Glo Assay Reagent (equilibrated to room temperature 20-25°C). Mix.
5. Transfer to a solid white 96-well plate. (No transfer is needed if the cells have been incubated in a TC-treated clear bottom white plate.)
6. Read luminescence with a luminescence plate reader at least two minutes after the reagent loading.
7. Collect data.

Attention: Steps 3-5 should be done at room temperature, 25°C. All data should be collected within 15 minutes of loading the reagents.

IV. References

Conway, S., *et al*., Characterisation of human melatonin mt(1) and MT(2) receptors by CRE-luciferase reporter assay. Eur J Pharmacol, 2000. 390(1-2): p. 15-24.

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:
F12 ham's: 90%, FBS: 10%, Penicillinum:100µg /ml, Strep:100 µg/ml.

Freezing Medium:

Complete culture medium: 45%, FBS: 45%, DMSO: 10%

Thawing Cells:

1. Quickly thaw frozen cells in a 37°C water bath, agitating continuously.
2. Using a 1 ml pipette, slowly pippet the cells up and down five times and add, drop by drop, to a 15 ml centrifuge tube containing 9 ml of fresh prewarmed complete F12 ham's medium. Then centrifuge at 1,000 rpm for five minutes.
3. Discard the supernatant medium and resuspend the cell pellet in 10 ml of fresh prewarmed complete F12 ham's medium. Transfer cells to a 10cm dish and incubate at 37°C with 5% CO_2. The recovery rate for frozen cells is usually 90% or above.

Subculturing:
When the cells reach 90% confluence, they need split. This cell line is normally split thrice weekly at 1:3 dilutions.

1. Carefully aspirate all the media. Gently rinse the cell layer with appropriate amount of 0.05% trypsin-EDTA, and aspirate it off.
2. Wait for about 1-3 minutes. Then dislodge the cells by gently tapping the sides of 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 F12 ham's medium (37°C) into a 10cm dish (10 ml for a 10cm dish).

Freezing Cells:

1. Repeat the steps 1-3 of subculturing section.
2. Centrifuge down the cells at 1,000 rpm for 5 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. F12 ham's: F-12 Nutrient Mixture(Ham), powder (Gibco BRL, Cat #21700-018)
2. FBS: Fetal Bovine Serum (GIBCO, Cat #26140)
3. Penicillin-Streptomycin,liquid (Gibco BRL, Cat # 15070-063)
4. G418 Sulfate, cell culture tested. (CALBIOCHEM, Cat #345810)
5. 0.05%Trypsin-EDTA: (Gibco BRL, Cat # 25300)
6. DMSO: dimethyl sulphoxide, for molecular biology (AMRESCO, Cat #0231)
7. Bright-Glo™ Luciferase Assay System (Promega, Cat. # E2620),
8. Venor®GeM Mycoplasma Detection kit: Minerva Biolabs Cat #11-1050
9. 10cm dish: Corning Cat #430167)
10. Cryogenic Vial: (Corning Cat #430659)
11. 96 Well White Round Bottom Polypropylene Not Treated Microplate (Corning, 3355)

Media and Solutions:

1. PBS (for preparation of 500 ml)

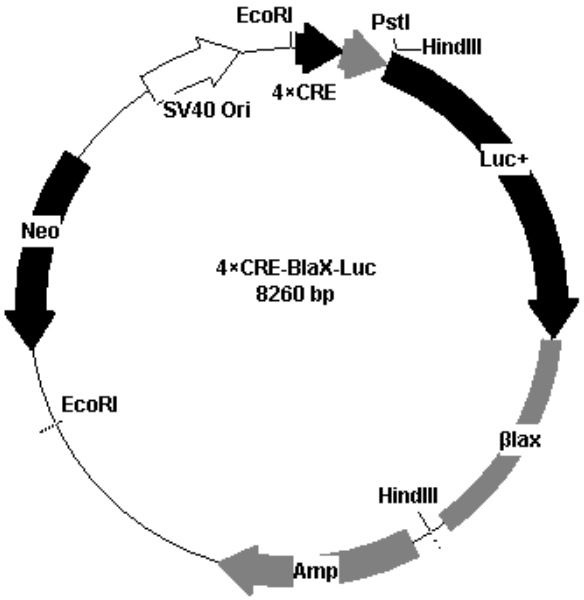
1)	KCl:	0.1 g
2)	KH_2PO_4:	0.1 g
3)	NaCl:	4.0 g
4)	$Na_2HPO_4.12H_2O$:	1.4 g

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 room temperature.

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 F12 ham's.
   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 4xCRE-βla-Luc

Name：**4xCRE-βla -Luc**
Plasmid Map：



GenScript Corporation
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: http://www.genscript.com

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Catalog Number: M00145

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