DATA SHEET

p53 Luciferase Reporter RKO Stable Cell Line

Catalog Number SL-0007 **(For Research Use Only)**

Introduction

The p53 pathway plays a crucial role in effective tumor suppression because of its central function in cell cycle regulation, DNA repair, cellular senescence, and apoptosis, which can be used for potentially develop new drug therapies against cancer. Upon activation by DNA damage, oncogene activation, or hypoxia, p53 binds to its DNA recognition site on the promoter regions of the target genes and regulate the gene expression. Signosis has established p53 luciferase reporter stable cell line, in which luciferase activity is specifically associated with the activity of p53. Therefore, the cell line can be used as a reporter system for monitoring the activation of p53 triggered by stimuli treatment, enforced gene expression and gene knockdown.

Principle of the assay

The cell line was established by transfection of p53 luciferase reporter vector along with G418 expression vector followed by G418 selection. The G418 resistant clones were subsequently screened for etoposide-induced luciferase activity. The clone with the highest fold induction (50 fold) was selected and expanded to produce this stable cell line.

Materials provided

- One vial of 5 X 10^6 cells, at passage 2, in Freezing Media (store the vial in liquid nitrogen until it is ready to be thawed).

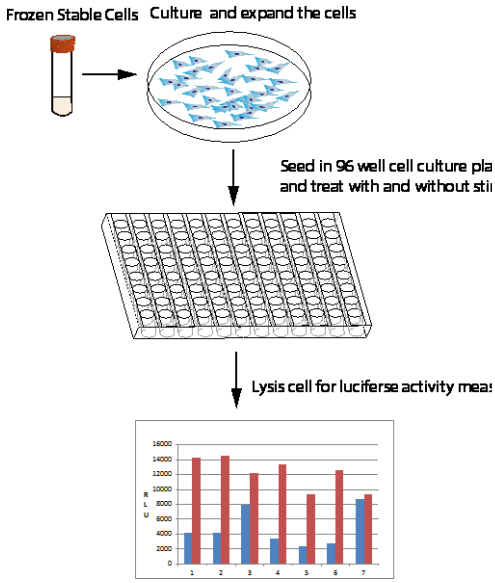

Fig. 1 Stable cell line diagram

Material required but not provided

- Dulbecco's Modified Eagle's Medium (DMEM)
- Fetal Bovine Serum (FBS)
- Penicillin (10,000 units/ml)
- Streptomycin (100ug/ml)
- G418 (Life Technologies)
- Freezing media
- Luciferase reporter system (Promega E-1500)

Handling cells upon arrival

- It is strongly recommended that you propagate the cells by following instructions as soon as possible upon arrival.
- Genetic instability is common in all transfected cells, therefore it is critical to prepare frozen stocks at early passages.
- Prepare **Complete Growth Media:**
  DMEM (in high glucose + sodium pyruvate + L-glutamine + Phenol Red) + Penicillin (100 units/mL) Streptomycin (100ug/ml) + 10% FBS + G418 (75ug/ml)

Initial Culture Procedure

Important: The first propagation of cells should be for generating stocks for future use. Cells undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

1. Quickly thaw cells in a 37°C water bath with careful agitation.
2. Add 10 ml Complete Growth Media to a sterile 15 ml centrifuge tube then add entire contents of the vial to the media.
3. Spin at 2,000 rpm for 5 minutes.
4. Discard supernatant.
5. Add 1ml of Complete Growth Medium to suspend pellet.
6. Add 10ml of Complete Growth Medium to culture dish and transfer resuspended pellet to culture dish containing Complete Growth Medium.
7. Pipette cells up and down to ensure the transfected cells are mixed well in the medium.
8. Place the culture dish with cells in a humidified incubator at 37°C with 5% CO2.
9. Change media every 2-3 days using Complete Growth Media.
10. When cells reach 90% confluency (usually within 1 week), prepare frozen stocks and continue propagate the rest of the culture for future assays.
11. Transfer vials from -80°C to liquid nitrogen for long term storage.

Prepare frozen stocks

1. Carefully remove the culture media from cells by aspiration.
2. Rinse cells with PBS, being careful to not dislodge attached cells. Then remove PBS by aspiration.
3. Add 2ml of 0.25% Trypsin/0.53mM Tris-EDTA solution to the culture dish.
4. Let the dish incubate with Trypsin for a few minutes or until cells have detached. Confirm detachment by observation under the microscope.
5. Add 10ml of Complete Growth Media and gently pipette up and down to break the clumps.
6. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 2,000 rpm for 5 minutes to collect the cells.
7. Aspirate the culture media and resuspend cells at a density of 5 x 10^6 cells/mL in freezing media.
8. Aliquot 1ml cells into cryogenic vials.
9. Place vials in a freezing container and store at -80°C overnight. Transfer to liquid nitrogen for long term storage.

Assay procedure

The following procedure should be followed as a guideline. You will need to optimize the assay conditions based upon your experimental set up.

1. The day before performing the assay, trypsinize the cells and plate each well of a 96-well plate with 5 X 10^4 cells in 100ul.
2. Incubate the plate in a humidified incubator at 37°C with 5% CO2 overnight.
3. Prepare inducing reagent at the optimal concentration in a 10ul volume.
4. Add inducing reagent directly to each well and incubate for an appropriate time to produce maximal induction.
5. Remove the media by aspiration and add 50ul lysis buffer to each well.
6. Incubate cells in lysis buffer for a few minutes at room temperature.
7. Rock culture dish several times to ensure complete coverage of the cells with lysis buffer. Pipette up and down to ensure complete lysis of cells.
8. Perform one freeze-thaw cycle at -80°C and room temperature.
9. Gently pipet up and down 2-3 times to mix.
10. Transfer 20ul of each lysate to a new 96-well plate for the luciferase assay.
11. Add 100ul of luciferase substrate to each well and gently pipette up and down.
12. Immediately read the plate in a luminometer.

Data Example

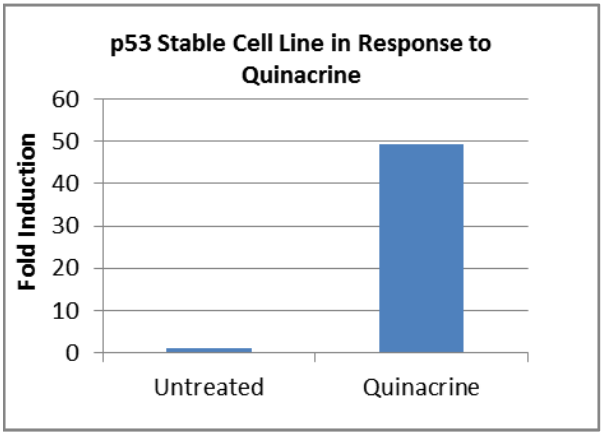

Fig. 2 Analysis of p53 Pathway Reporter RKO Cell Line in response to stimuli.
The cells were seeded on a 96-well plate for overnight with DMEM including 10% FBS. The cells then were treated with or without 2ug/ml Quinacrine respectively in DMEM and 0.1% FBS for 16 hours.