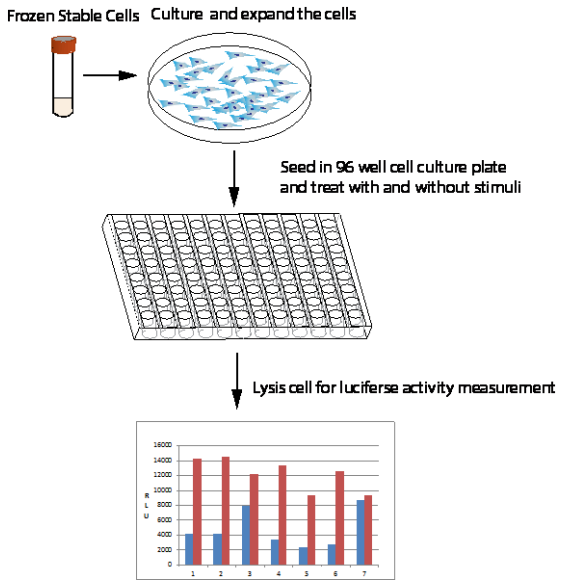
DATA SHEET

TNFa/NFκB Pathway Luciferase Reporter HeLa Stable Cell Line

Catalog Number SL-0001 **(For Research Use Only)**

Introduction

NFkB plays a critical role in the regulation of a large variety of genes involved in immune and inflammatory responses, developmental processes, cellular growth, and apoptosis. It can be activated by some cytokines, such as TNFa. TNFa is a proinflammatory cytokine that can activate and regulate NFkB pathway. When activated by TNFa, NFkB translocates into nucleus, where NFkB binds to its response element on the promoter region and regulates a wide spectrum of gene expression. Dysfunction of TNFa induced NFkB pathway is associated with cancer, inflammatory and autoimmune diseases, and viral infection. Therefore, monitoring the NFkB pathway is essential to unveil the mechanism of these diseases and conduct drug discovery. Signosis has established TNFa/NFkB Pathway Luciferase Reporter Hela Stable Cell Line, in which NFkB activity can be represented by luciferase activity. Therefore, the cell line can be used as a reporter system for monitoring NFkB triggered by stimuli treatment, enforced gene expression and gene knockdown.



Stable cell line diagram

Principle of the assay

The cell line was established by transfection of NFkB luciferase reporter vector along with hygromycin expression vector followed by hygromycin selection. The hygromycin resistant clones were subsequently screened for TNFa-induced luciferase activity. The clone with the highest fold induction (100 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).

Material required but not provided

- Dulbecco's Modified Eagle's Medium (DMEM)
- Fetal Bovine Serum (FBS)
- Penicillin (10,000 units/ml)
- Hygromycin B (Roche)
- 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 a common in all transfected cells, therefore, it is critical to prepare numbers of 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 + Hygromycin (100ug/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 ^{o}C water bath with careful agitation.
2. Transfer entire contents of the vial to a sterile 50 ml centrifuge tube and add 10 ml of cold Complete Growth Media to cells.
3. Spin at 3,000 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 suspended 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 ^{o}C or 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 to liquid nitrogen for long term storage.

Prepare frozen stocks

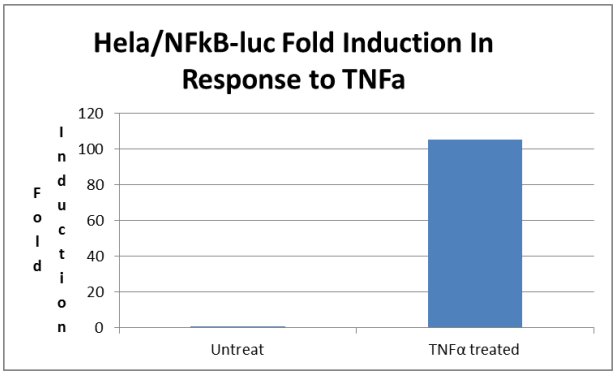
1. Carefully remove the culture media from cells by aspiration.
2. Rinse cells with PBS, being careful to not dislodge attached cells. Then remove PBS by aspiration.
3. Add 2ml of 0.25% Trypsin/0.53mM Tris-EDTA solution to the culture dish.
4. Let the dish incubate with Trypsin for a few minutes (2-3 min). Confirm detachment by observation under the microscope.
5. Add 10ml of pre-warmed 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 125 x g 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^{o}C overnight.

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 ^{o}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^{o}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



The Hela/NFkB stable cells were seeded in 96 well plate and incubated for overnight. The cells then were treated with or without 20ng/ml TNFa for 8 hours. The cells were lysed and subject to measurement of luciferase activity.