DATA SHEET

NF-κB/293/GFP-Luc™ Transcriptional Reporter Cell Line

Cat. # TR860A-1

User Manual

Store vial in vapor phase of liquid nitrogen on receipt

Contents

I. Introduction and Background

A. Overview

The NF-κB/293/GFP-Luc™ cell line was specifically designed for monitoring the NF-κB signal transduction pathway *in vitro*. The unique feature of this reporter cell line allows researchers to monitor the NF-κB pathway by the detection of GFP fluorescence as well as Luciferase for quantitative transcription activation reporter assays. The NF-κB/293/GFP™ cells were derived from System Biosciences' 293 TN Producer Cell Line (Cat. # LV900A-1). 293 TN cells were transduced with HIV-based pseudoviral particles packaged with a lentivector that co-expresses destabilized copGFP and Firefly Luciferase reporter driven by the minimal cytomegalovirus (mCMV) promoter in conjunction with four copies of the NF-κB consensus transcriptional response element upstream of mCMV (Figure1). Positively transduced cells were cloned by Fluorescent Activated Cell Sorting (FACS), and monoclonal populations that stably retained the proviral expression construct in 293 TN cell genomic DNA over many passages were selected. Clones that demonstrated a robust (≥ 250-fold) increase in GFP expression upon stimulation with TNF-α at 10 ng/ml for 24 hours were chosen for further development of the cell line. The resulting NF-κB/293/GFP-Luc™ cell line is the monoclonal population that exhibited the strongest response to TNF-α stimulation (Figure 2 and 3,) and retained all desirable attributes of the parental 293 TN cells (superior adherence, growth rate, morphology, and neomycin resistance).

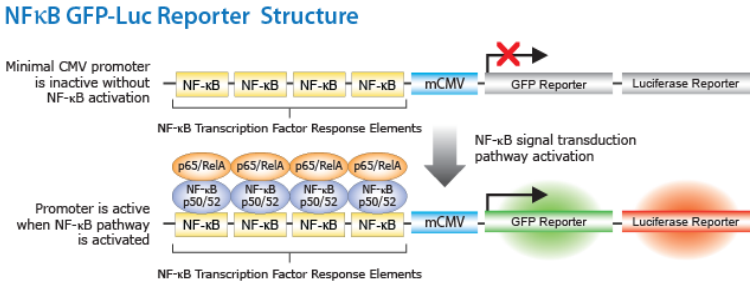


Figure 1: NF-kB/GFP-Luc Reporter Structure

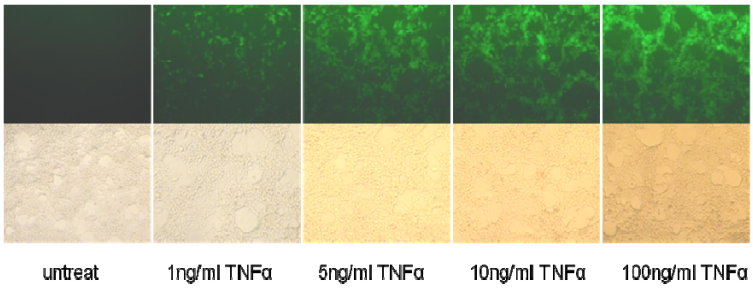


Figure 2: NF-kB/293/GFP-Luc™ cell line Epifluorescence Microscopy
NF-kB/293/GFP-Luc™ cells were treated with the indicated amount of TNFα for 18 hours.

Utilization of the GFP reporter gene allows the researcher to detect NF-κB activation by fluorescence microscopy, and offers the advantage of allowing for GFP-positive or negative cells to be sorted by FACS, while Firefly Luciferase provides a familiar, well-established and quantitative assessment of transcription activation. As a result, the NF-κB/293/GFP-Luc™ cell line is completely compatible with SBI's HIV- and FIV-based genome-wide siRNA libraries and individual siRNA lentiviral vectors for RNAi knockdown studies, as well as cDNA vectors to identify genes involved in the stimulation or inhibition of the NF-κB pathway. NF-κB/293/GFP-Luc™ cells are highly transfectable and transducible human cell lines which serve as useful *in vitro* cell models for a variety of research applications, including screening of small molecule inhibitors or activators of the NF-κB pathway, and the identification of genes involved in the inhibition or activation of the pathway by use of genome-wide siRNA libraries or specific siRNAs, available from System Biosciences. For an example of an experiment in which SBI's Human 50K GeneNet™ siRNA Library was used in conjunction with the NF-κB/293/GFP-Luc™ cell line to screen for genes involved in the activation or inhibition of the NF-κB signal transduction pathway.

B. NF-κB Signal Transduction Pathway

NF-κB, a member of the rel family of transcription factors, regulates several important physiological processes, including immune responses, inflammation, cell growth, apoptosis, tumorigenesis, and the expression of certain virus genes (HIV and CMV). As a result, the NF-κB signaling pathway has been a target for pharmacological intervention, especially in models of inflammation or cancer, where the pathway is often constitutively active (1). Over 750 inhibitors of the NF-κB pathway have been identified, including both natural and synthetic molecules (1). Conversely, many different stimuli have been identified which activate the NF-κB pathway, including cytokines such as TNF-α and interleukin-1β, pathogenic bacteria and viruses, bacterial lipopolysaccharide and peptidoglycan, and oxidative stress. The NF-κB/293/GFP-Luc™ cell line allows the researcher to study both potential inhibitors, activators, and antagonists of the NF-κB pathway, and as such, is an extremely valuable tool for a wide variety of research applications.

C. List of Components

NF-κB/293/GFP-Luc™ cells (catalog #) are supplied in one vial containing approximately 2× 10^6 cells in 1 ml of freezing medium (FBS w/ 5% Dimethyl sulfoxide, DMSO).

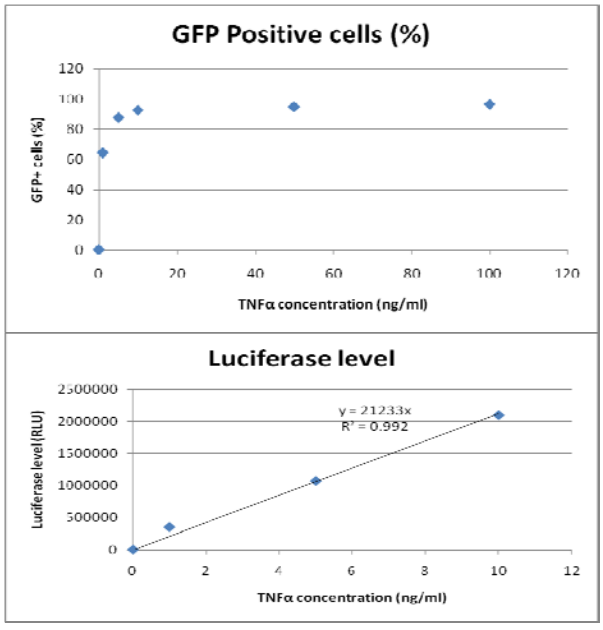
1 ml NF-κB/293/GFP-Luc™ cells (~2 × 10^6 cells)

D. Shipping and Storage

The NF-κB/293/GFP-Luc™ cell lines are shipped frozen on dry ice. It is strongly recommended that the NF-κB/293/GFP-Luc™ cells be thawed and propagated as soon as possible following receipt (see "Thawing NF-κB/293/GFP-Luc™ cells" protocol below). If long-term storage of the frozen cells is required, place vial in the vapor phase of liquid nitrogen. Storage of the cells directly in liquid nitrogen requires use of protective tubing, such as Nunc™ Cryoflex™ Tubing (Mfr. No. 373958). Storage of the cells at -80ºC is suitable only for short periods of time (3-4 days), and may result in loss of viability and is not recommended.

E. Product Qualification

Each lot of NF-κB/293/GFP-Luc™ cells is tested for growth and viability following recovery from cryopreservation. In addition, each lot is tested for expression of Green Fluorescent Protein (GFP) reporter gene and the activatity of Luciferase reporter following stimulation with 10 ng/ml TNF-α(Figure 2 and 3) . Only those cells exhibiting ≅ 300-fold average GFP expression compared to unstimulated 293-kappa B cells pass quality assurance (Figure 4,).



(A)FACS data

(B) Luciferase assay

Figure 3 : Activation of NFkB/293/GFP-Luc™ cells with increasing amounts of TNF-α.
NFkB/293/GFP-Luc™ cells were plated at a concentration of 1 million cells/ml into each well of a 24-well plate. TNF-α was added in the amount indicated in the Figure. (A) GFP reporter induction was analyzed by flow cytometry, and the percentage of GFP positive cells were plotted against the amount of TNF-α. (B) Luciferase assay was performed to show the Luciferase activity induction. The Luciferase level were plotted against the amount of TNF-α.

Flow cytometry analysis of NFkB/293/GFP-luc™ cells treated with 10 ng/ml TNF-α vs. untreated cells.

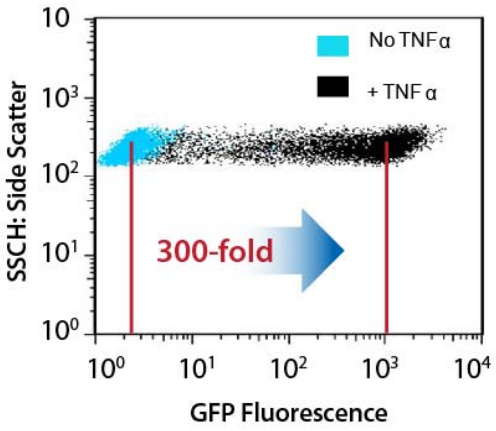


Figure 4 : Flow cytometry analysis of NFkB/293/GFP-Luc™ cells treated with 10 ng/ml TNF-α vs. untreated cells.

F. Safety Guidelines

The NF-κB/293/GFP-Luc™ cell line falls within NIH Biosafety Level 2 criteria and should be handled as potentially biohazardous material. This product contains Dimethyl sulfoxide (DMSO), a hazardous material. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm. It is also important to check with the health and safety guidelines at your institution regarding the use of potentially biohazardous cell lines, and always follow standard tissue culture practices, which include:

- Wearing gloves, safety glasses, and a lab coat at all times when conducting the procedure
- Always working with cells in a Class II laminar flow hood
- Carefully performing all procedures to minimize the creation of aerosols or splashes
- Decontamination of all work surfaces at least once a day and immediately after working with cells

Decontamination of all cultures, viral stocks, and other regulated wastes before disposal by an approved method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (“Biohazard”, “infectious waste”) container and sealed for transportation from the laboratory.

II. Protocols

A. Required Media for NF-κB/293/GFP-Luc™ cells

The table below shows the recommended complete medium and freezing medium for maintenance of NF-κB/293/GFP-Luc™ cells.

Complete Growth Medium	Freezing Medium
D-MEM, high gLucose	90% Complete Growth Medium
10% fetal bovine serum (FBS)*	10% DMSO
2 mM L-glutamine	
1% Penicillin/Streptomycin (10,000 I.U. Penicillin; 10,000 μg/ml Streptomycin)	

*FBS does not require heat inactivation to be used with the NF-κB/293/GFP-Luc™ cell line

B. Thawing Cells

Use the following protocol to thaw NF-κB/293/GFP-Luc™ cells to initiate the culture. The initial propagation of cells should be used to generate stocks to be frozen and stored for future use.

1. Remove the frozen vial of cells from liquid nitrogen and quickly thaw them by swirling in a 37ºC water bath. Try to keep the O-ring and cap of the vial out of the water, to prevent possible contamination. Wear eye protection.
2. Before cells are completely thawed, remove from 37ºC water bath and decontaminate outside of the vial with 70% ethanol.
3. Using sterile technique, transfer the cells to a T-75 cm^2 tissue culture flask containing 20 ml of complete medium at room temperature. Transfer entire contents of the vial to the T-75 flask, and do not pipet cells up and down as this may kill the cells.
4. Swirl the T-75 flask to evenly distribute cells. Incubate the flask at 37ºC, 5% CO_2 overnight to allow cells to attach to the bottom of the flask.
5. The following day, pour off or aspirate off medium and replace with 20 ml fresh complete medium at room temperature.
6. Incubate the cells at 37ºC, 5% CO_2 and check daily until they reach 85-95% confluency (about 2-4 days).
7. Once NF-κB/293/GFP-Luc™ cells reach 85-95% confluency, subculture the cells as described below. For the initial culture, it is recommended to archive several frozen stocks and continue to propagate remainder of cells for use in experiments.

Note: Vials inappropriately stored directly in liquid nitrogen without protective tubing, such as Nunc™ Cryoflex™ Tubing, may contain liquid nitrogen. Upon thawing, the liquid nitrogen will quickly convert to the gas phase and may cause the vial of cells to explode. This is a very hazardous situation, and should only be performed using protective gloves and clothing, and a full-face mask. To avoid this situation, store vials only in the vapor phase of liquid nitrogen or use the protective tubing described above if the vial must be stored directly in the liquid phase of liquid nitrogen.

C. Subculturing Cells

When NF-κB/293/GFP-Luc™ cells reach 85-95% confluency, they are ready to be subcultured, or transferred to a new tissue culture flask. This is typically every 2-3 days. Use the following protocol to subculture NF-κB/293/GFP-Luc™ cells grown in a T-75 cm^2 flask. If a different sized tissue culture flask is being used, scale the reagent and media volumes accordingly.

1. Remove complete medium from the flask by pouring or aspiration. Wash the cells once with 5 ml PBS to remove excess medium, and discard PBS. Complete medium containing FBS will inhibit trypsin.
2. Add 5 ml of pre-warmed (room temperature to 37ºC) trypsin-EDTA (0.5% trypsin with EDTA 4Na) solution to the cell monolayer and incubate for 5 minutes at 37ºC, 5% CO_2, or until cells detach. If cells are still attached after 5 minutes, swirl the flask gently and incubate a few minutes longer.

3. Add 5 ml of complete medium and *gently* pipet up and down to break up cell clumps and achieve a suspension of single cells. Transfer the cell suspension to a 15 ml sterile, conical centrifuge tube.
4. Determine viable and total cell counts by use of a hemocytometer chamber or a Coulter Counter.
5. Dispense 1 ml of the cell suspension into each new T-75 cm^2 flask containing 20 ml of pre-warmed medium. This is a 1:10 split (1/10) of the original cell population. Cells should be 85-95% confluent after 2 to 3 days. If using a culture flask other than a T-75 cm^2, scale the volume of cell suspension used. If cells are to be used for an experimental assay, seed cells at the required density for the experiment.
6. Incubate cells at 37ºC, 5% CO_2 until 85-95% confluent and subculture again, or incubate until they reach the desired confluency for the experiment.

D. Freezing Cells

Preparation of frozen cell stocks

Before beginning the freezing protocol below, label all cryovials and prepare freezing medium (complete growth medium with 10% DMSO). Keep freezing medium at 4ºC or on ice until ready for use.

1. Culture a T-75 cm^2 flask of NF-κB/293/GFP-Luc™ cells to 85-95% confluency.
2. Remove cells from the flask by following steps 1 through 5 in "Subculturing NF-κB/293/GFP-Luc™ cells", above.
3. Centrifuge the remaining cell suspension at 250 × g for 10 minutes at room temperature. Aspirate the medium from the cells and resuspend the pelleted cells in 1 ml of freezing medium for every 1 ml of original cell suspension (*e.g.*, if cells retrieved from the original T-75 cm^2 flask were resuspended in 10 ml and 1 ml was used for subculturing, centrifuge the remaining 9 ml of cells, aspirate medium, and resuspend in 9 ml of freezing medium. If more or less cell suspension was used, adjust the volume of freezing medium accordingly). Each T-75 cm^2 flask at 85-95% confluency will yield approximately ten (10) 1 ml aliquots for freezing.
4. Dispense 1 ml aliquots of the cells into cryovials following manufacturer's recommendations.
5. Freeze cells using either a controlled-rate freezing apparatus or manually using a freezing container. The apparatus should provide a controlled freezing rate of 1ºC/minute. Cells should be frozen to -70ºC to -80ºC overnight.
6. Transfer frozen cell stocks to liquid nitrogen storage the following day.

III. References

1. **Egan, L. J. and Toruner, M.** NF-κB Signaling: Pros and Cons of Altering NF-κB as a Therapeutic Approach. 2006; Ann. N.Y. Acad. Sci. 1072: 114–122.