Description

The NF-κB reporter (Luc)-NIH-3T3 cell line is designed for monitoring nuclear factor Kappa B (NF-κB) signal transduction pathways. It contains a firefly luciferase gene driven by four copies of the NF-κB response element located upstream of the minimal TATA promoter. After activation by pro-inflammatory cytokines or stimulants of lymphokine receptors, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter gene.

Application

- Monitor NF-κB signaling pathway activity.
- Screen for compound activity of the NF-κB signaling pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

NIH/3T3

Mycoplasma Testing

The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 5	BPS Bioscience #60182
Growth Medium 5A	BPS Bioscience #79534

Materials Required for Cellular Assay

Name	Ordering Information
hTNFα	R&D Systems 210-TA
QNZ (EVP4593) or other suitable inhibitor control	Selleckchem #S4902
Assay Medium: Thaw Medium 5	BPS Bioscience #60182
Growth Medium 5A	BPS Bioscience #79534
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
ONE-Step™ luciferase assay system	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 5A.

Media Required for Cell Culture

Thaw Medium 5 (BPS Bioscience #60182):
DMEM (Hyclone #SH30243.01), supplemented with 10% Bovine Calf Serum (Hyclone #SH3007203), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 5A (BPS Bioscience #79534):
Thaw Medium 5 (BPS Bioscience Cat. #60182) and 600 µg/ml of Geneticin (Life Technologies #11811031).

Assay Medium: Thaw Medium 5 (BPS Bioscience #60182)

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 5 (**no Geneticin**).
2. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 5 (**no Geneticin**).
3. Transfer the resuspended cells to a T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. At first passage, switch to Growth Medium 5A (**contains Geneticin**).

Cell Passage

1. To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 5A and seed appropriate aliquots of cell suspension into new culture vessels.
2. Subcultivation ratio: 1:5 to 1:10, twice a week.

Cell Freezing

1. To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. Add Growth Medium 5A and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS).
3. Place cells in a reduced rate freezing box (-0.5°C to -1°C per minute) down to overnight, then move cells to liquid nitrogen for long term storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Functional Validation and Assay Performance

The following assays are designed for a 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

A. TNFα dose response

1. Harvest NF-κB reporter (Luc)-NIH-3T3 cells from culture in Growth Medium 5A and seed cells at a density of 25,000 cells per well into white opaque 96-well microplate in 50 µl of assay medium. Incubate at 37°C with 5% CO_2 overnight.
2. Prepare serial dilutions of TNFα at 2x in assay medium. Add 50 µl of TNFα to the cells.
3. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
4. Incubate at 37°C with 5% CO2 for 5-6 hours.
5. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

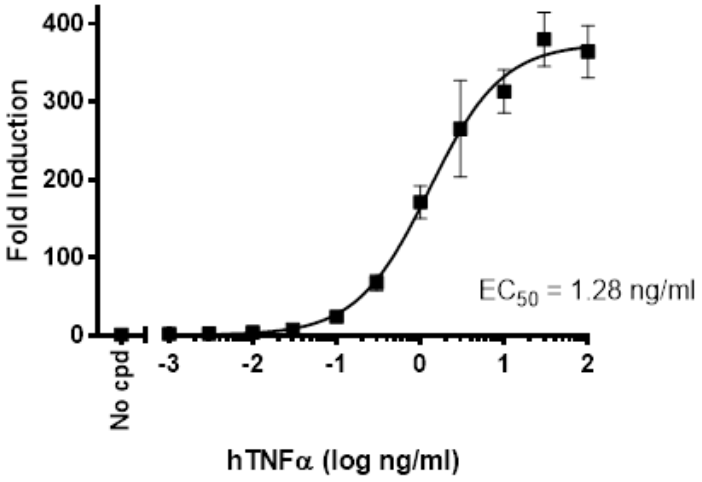

Figure 1. TNFα dose response in NF-κB reporter (Luc)-NIH-3T3 cells. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without TNFα treatment.

B. Testing Inhibitors

1. Harvest NF-κB reporter (Luc)-NIH-3T3 cells from culture in Growth Medium 5A and seed cells at a density of 25,000 cells per well into white opaque 96-well microplate in 40 µl of assay medium. Incubate at 37°C with 5% CO_2 for 6 hours.
2. Prepare serial dilutions of test compounds or QNZ control at 2x in assay medium. Add 50 µl of dilutions to cells.
3. Add 50 µl of assay medium to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).
4. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
5. Incubate at 37°C with 5% CO_2 overnight.
6. Prepare hTNFα at 10x in assay medium. Final concentration on the cells: TNFα = 10 ng/ml. Add 10 µl of diluted hTNFα to the wells with test inhibitors. Add 10 µl of assay medium to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).
7. Incubate at 37°C with 5% CO_2 for 6 hours.
8. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

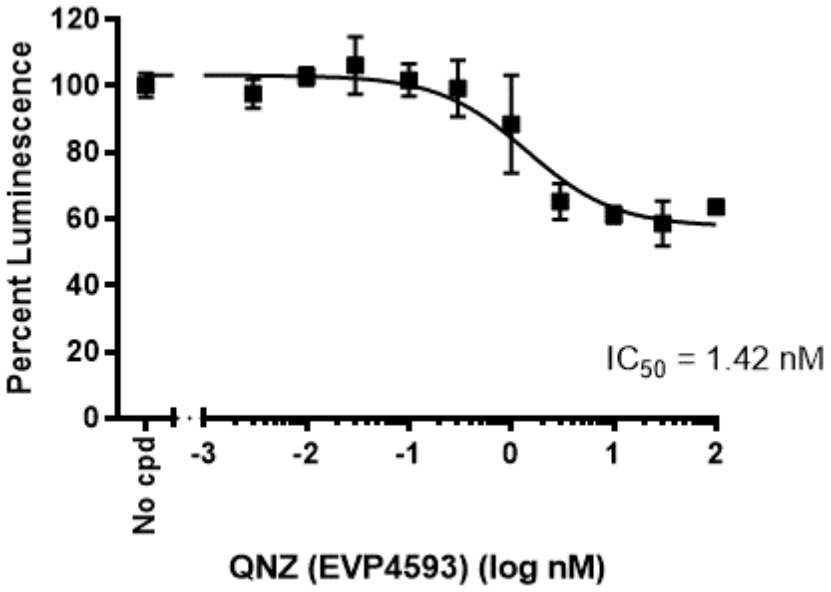

Figure 2. QNZ (EVP4593) inhibition of hTNFα stimulation in NF-κB reporter (Luc)-NIH-3T3 cells. The results are shown as percent luminescence of luciferase reporter expression. Percent luminescence was determined by comparing values against the mean value for control cells.

License Disclosure

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

References:

1. Pessara U, Koch N (1990) Tumor necrosis factor alpha regulates expression of the major histocompatibility complex class II-associated invariant chain by binding of an NF-κb-like factor to a promoter element. *Mol Cell Biol*. **10(8):**4146-4154.
2. Baeuerle PA (1998) Pro-inflammatory signaling: last pieces in the NF-κb puzzle? *Curr Biol*. **8(1):**R19-R22.

Related Products

Products	*Catalog #*	*Size*
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB reporter (Luc) - Jurkat Cell line	60651	2 vials
NF-κB reporter (Luc) - CHO-K1 Cell line	60622	2 vials
NF-κB reporter (Luc) – A549 Cell line	60625	2 vials
ONE-StepTM Luciferase Assay System	60690	Multiple Sizes
NF-κB Reporter Kit (NF-κb Signaling Pathway)	60614	500 reactions
Transfection Collection™ : NF-κB Transient Pack (NF-κB Signaling Pathway)	79268	500 reactions
Transfection Collection™ : NF-κB Reporter Cellular Assay Pack (HEK293)	79327	2 vials
Transfection Collection™ : NF-κB Reporter Cellular Assay Pack (HCT116)	79326	2 vials