Description

NF-κB luciferase reporter construct is stably integrated into the genome of A549 cells. The firefly luciferase gene is controlled by 4 copies of NF-κB response element located upstream of the TATA promoter. Following activation by stimulants, endogenous NF-κB transcription factors bind to the DNA response elements to induce transcription of the luciferase gene.

Background

NF-κB signaling plays a pivotal role in regulating cell development and immune homeostasis. Activation of NF-κB through tumor necrosis factor receptors (TNFR) or the TNFR superfamily member CD40 occurs upon engagement with their respective ligands TNFα or CD40L. Activation of NF-κB enhances cell inflammation and prevents apoptosis, which contribute to tumor development. The A549 lung epithelial cell line is ideal in an in vitro lung disease model for high throughput screening of oncogene inhibitors upstream of the NF-κB signaling pathway.

Application

The NF-κB-luciferase / A549 cell line is suitable for monitoring the activity of NF-κB transcription factor through luminescence readout instead of using electrophoretic mobility shift assay (EMSA). It provides a platform to enable study of a plethora of signaling pathways upstream of NF-κB in the context of cancer immunology and infectious diseases. NF-κB stimulation by cytokines including TNFα and IL-1ß have been validated for this cell line.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~3 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

Human alveolar vassal carcinoma cell line. Adherent epithelial cells.

Vector

NF-κB-Luciferase was cloned into pcDNA3.1™ (+) vector (Invitrogen, Cat. No. V79020).

Mycoplasma Testing

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Cat. #LT07- 118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Cat. #LT07-518) was used as a positive control.

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 6	BPS Bioscience #60183
Growth Medium 6B	BPS Bioscience #79657

Materials Required for Cellular Assay

Name	Ordering Information
hTNFα	R&D Systems 210-TA
IKK-16 dihydrochloride or other suitable inhibitor control	Sigma SML 1138
Assay Medium: Thaw Medium 6	BPS Bioscience #60183
Growth Medium 6B	BPS Bioscience #79657
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 6B.

Media Required for Cell Culture

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium (Hyclone #SH30243.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 6B (BPS Bioscience #79657):
Thaw Medium 6 (BPS Bioscience, #60183) plus 1 mg/mL Geneticin®, G418 Sulfate (Thermo Fisher, Cat. No. 11811031).

Assay Medium: Thaw Medium 6 (BPS Bioscience #60183)

Cell Culture Protocol

Cell Thawing

1. Prepare a 15ml conical tube with 10 ml of pre-warmed Thaw Medium 6 (no Geneticin).
2. Quickly thaw cells in a 37°C water bath with constant and slow agitation.
3. Clean the outside of the vial with 70% ethanol and immediately transfer the entire contents to the conical tube.
4. Spin cells at 200 x g for 5 minutes, and remove all medium from the pellet.
5. Resuspend in 15 ml Thaw Medium 6 (no Geneticin) and transfer to a T-75 flask.
6. Gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2.
7. 24 hours after incubation, change culture to fresh Thaw Medium 6 (no Geneticin); avoid disturbing the attached cells.
8. Continue to monitor growth for 2-3 days and change the media to remove dead cell debris, if necessary.
9. Begin adding Growth Medium 6B after multiple cell colonies (in clumps) start to appear (indicative of healthy cell division).

Subculture:

1. When cells have reached 90% confluency, remove Growth Medium 6B and gently wash cells twice with PBS (without Magnesium or Calcium).
2. Treat cells with 1 ml of 0.25% tryspin/EDTA and incubate for 2-3 minutes at 37°C.
3. Dispense 9 ml of pre-warmed Growth Medium 6B to trypsinized cells and gently pipette up and down to neutralize trypsin and break apart any cell clumps.
4. Dispense 1 ml of cell suspension into a new T-75 flask containing 9 ml of prewarmed Growth Medium 6B.
5. Incubate cells in a humidified 37°C incubator with 5% CO_2.

   Recommended split ratio: 1:10 twice per week.

Cryopreservation:

1. When cells reach 90% confluency, use trypsin to remove cells from plate, spin cells, and remove medium from the pellet.
2. Resuspend the cells in freezing medium (10% DMSO in FBS). Freeze cells using a reduced rate freezing box (-0.5°C to -1°C per minute) down to -80°C, then move cells to liquid nitrogen for long term storage.
3. Cells have been demonstrated to be stable for at least 15 passages; BPS Bioscience recommends preparing frozen stocks so cells are not used beyond passage 20.

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)-A549 cells from culture in Growth Medium 6B and seed cells at a density of 10,000 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% CO_2 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.

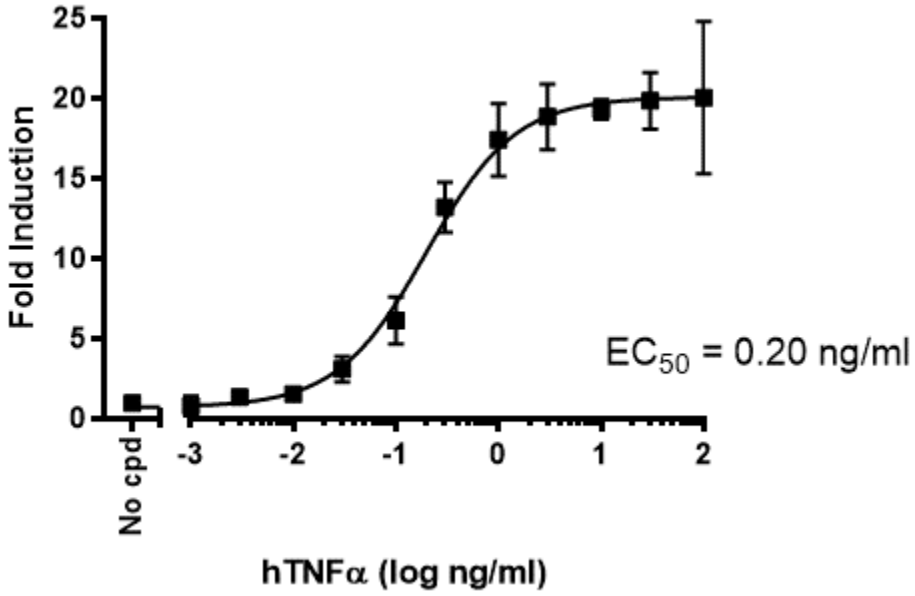

Figure 1. TNFα dose response in NF-κB reporter (Luc)-A549 cells. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without TNFα treatment.

B. Testing Inhibitors

1. Harvest NF-κB reporter (Luc)-A549 cells from culture in Growth Medium 6B and seed cells at a density of 10,000 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 IKK-16 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 = 1 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.

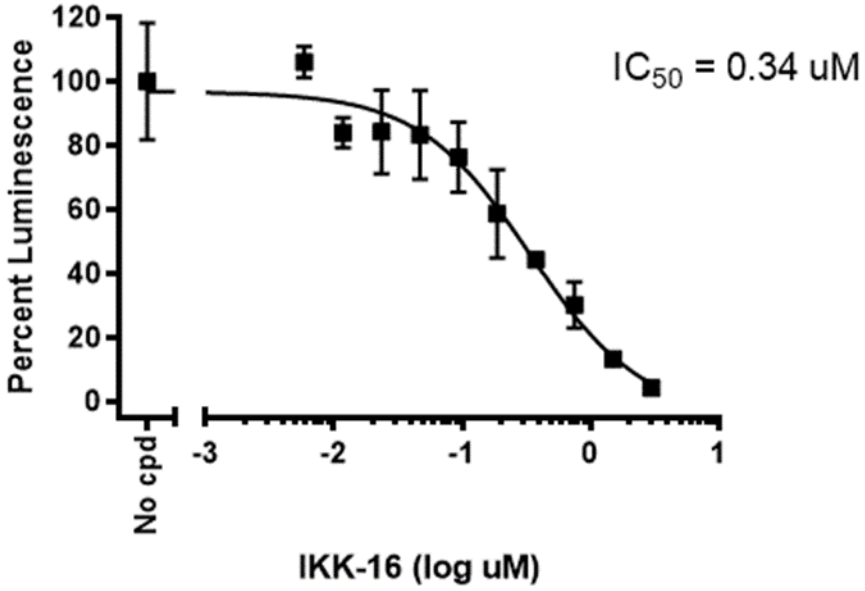

Figure 2. IKK-16 inhibition of hTNFα stimulation in NF-κB reporter (Luc)-A549 cells. The results are shown as percent luminescence of luciferase reporter expression. Percent luminescence was determined by comparing values against the mean value for control cells.

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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. Cherfilis-Vicini J. *et.al.* (2010) Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. *J. Clin. Invest*. **120**: 1285-1297.
2. Chen W. *et.al.* (2011) NF-κB, a mediator for lung carcinogenesis and a target for lung cancer prevention and therapy. *Front. Biosci.* **16:** 1172-1185.
3. Callister ME *et.al.* (2008) PMX464, a thiol-reactive quinol and putative thioredoxin inhibitor, inhibits NF-κB-dependent proinflammatory activation of alveolar epithelial cells. *Br. J. Pharm*. **155:** 661-672.
4. Schmeck B *et.al.* (2007) Legionella pneumophilia-induced NF-κB- and MAPK-dependent cytokine release by lung epithelial cells. *Eur. Respir. J.* **29:** 25-33

Related Products

Products	*Catalog #*	*Size*
ONE-Step™ Luciferase Assay System	60690	Multiple Sizes
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB reporter (Luc) - HCT116 Cell line	60623	2 vials
NF-κB reporter (Luc) - CHO-K1 Cell line	60622	2 vials
NF-κB reporter (Luc) – NIH/3T3 Cell line	79469	2 vials
NF- κB Reporter (Luc) – THP-1 Cell Line	79645	2 vials
NF- κB Reporter (Luc) – Raw 264.7 Cell line	79978	2 vials
NF-κB (GFP) – Reporter HEK293 Recombinant Cell Line	79402	2 vials
NF-κB reporter (Luc) Cell Based Assay Kit	60614	500 reactions
OX40 / NF-κB Reporter – HEK293 Recombinant Cell Line	60482	2 vials
CD137 (4-1BB) /NF-κB Reporter - HEK293 Cell Line	79289	2 vials
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	60621	2 vials
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line	60535	2 vials