Description

NF-κB Luciferase Reporter A549 Cell Line is an A549 cell line expressing the NF-κB (nuclear factor kappa0light-chain-enhancer of activated B cells) luciferase reporter. The firefly luciferase reporter 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 reporter. This cell line has been validated for NF-κB stimulation by TNFα (tumor necrosis factor α) and LIGHT (tumor necrosis factor superfamily member 14 or TNFSF14).

Background

NF-κB signaling plays a pivotal role in regulating cell development and immune homeostasis, by regulating DNA transcription, cytokine production and apoptosis. It is ubiquitously present in almost all mammalian cells, and responds to cellular stress signals, such as stress, free radicals, UV radiation and bacteria and virus. Activation of NF-κB through tumor necrosis factor receptors (TNFR) or the TNFR superfamily member Lymphotoxin-β (LTβR) occurs upon engagement with their respective ligands TNFα (tumor necrosis factor α) or LIGHT (tumor necrosis factor superfamily member 14 or TNFSF14). Activation of NF-κB enhances cell inflammation and prevents apoptosis, which contribute to tumor development. The A549 lung epithelial cell line is ideal as *in vitro* lung disease model for high throughput screening of inhibitors of upstream effectors of the NF-κB signaling pathway.

Application(s)

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

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

Human alveolar vassal carcinoma cell line. Adherent epithelial cells.

Mycoplasma Testing

The cell line has been screened 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 6	BPS Bioscience #60183
Growth Medium 6B	BPS Bioscience #79657

Materials Required for Cellular Assay

Name	Ordering Information
hTNFα (Carrier Free)	R&D Systems 210-TA
LIGHT, His-Tag (Human) Recombinant	BPS Bioscience #71266
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, the use of validated and optimized media from BPS Bioscience is *highly recommended* to. Note that using similar but not BPS Bioscience validated reagents can result in suboptimal performance.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 6B (BPS Bioscience #79657):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin.

Media Required for Functional Cellular Assay

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: A549 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 6.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 200 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 6.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 6 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Continue to monitor growth for 2-3 days and change the media to remove dead cell debris, if necessary.

6. Switch to Growth Medium 6B once multiple cell colonies (cell clumps) start to appear (indicative of healthy cell division).

7. Cells should be passaged before they are fully confluent (at 90% confluency). At first passage and subsequent passages, use Growth Medium 6B.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 6B, gently dissociate any clumps by pipetting up and down, and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 6B.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1/10 twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 6B and count the cells.

   Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

3. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

4. Transfer the vials to liquid nitrogen the next day 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.

Validation Data

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include "Stimulated Cells"/ "Test Inhibitor", "Background Control" and "Unstimulated Control" conditions.

A. NF-κB Luciferase Reporter A549 Cell Line Response to the TNF Superfamily agonists TNFα and LIGHT

1. Harvest NF-κB Luciferase Reporter A549 cells from culture in Growth Medium 6B and seed cells at a density of 10,000 cells per well in 50 µl of Assay Medium into a white opaque 96-well microplate. Leave empty wells as cell-free control wells ("Background Control").

2. Incubate at 37°C with 5% CO_2 overnight.

3. Prepare a serial dilution of agonist in Assay Medium at 2x the final testing concentrations (50 µl/well).

4. Add 50 µl of diluted agonist to the "Stimulated Cells" wells.

5. Add 50 µl of Assay Medium to the "Unstimulated Control" wells.

6. Add 100 µl of Assay Medium to "Background Control" wells (cell-free wells).

7. Incubate at 37°C with 5% CO_2 for 5-6 hours.

8. Add 100 µl of ONE-Step™ Luciferase reagent per well.

9. Incubate at Room Temperature (RT) for ~15 to 30 minutes.

10. Measure luminescence using a luminometer.

11. The "Background Control" luminescence value should be subtracted from all readings.

12. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

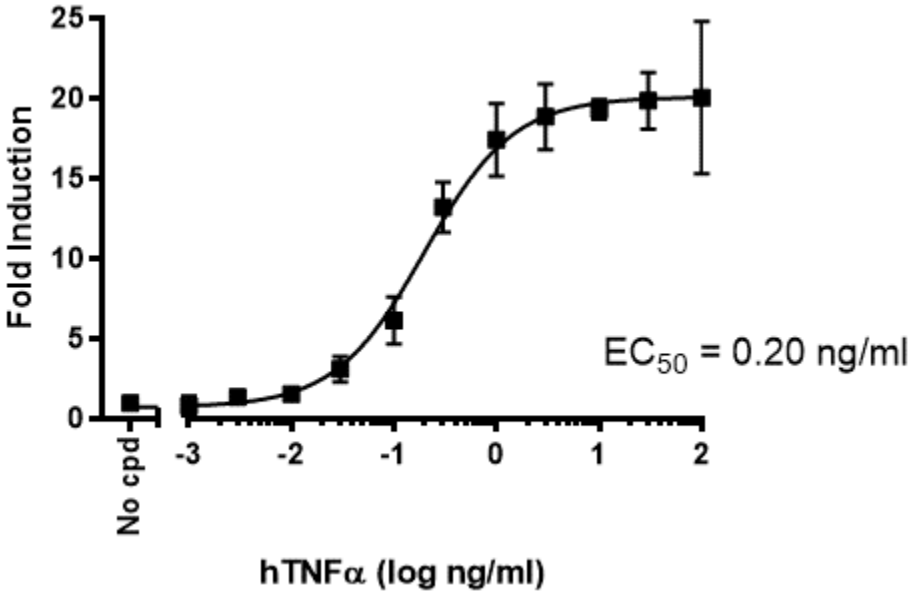

Figure 1. Dose response curve of NF-κB Luciferase Reporter A549 Cell Line to TNFα.
NF-κB Luciferase Reporter A549 cells were treated with increasing concentrations of TNFα. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

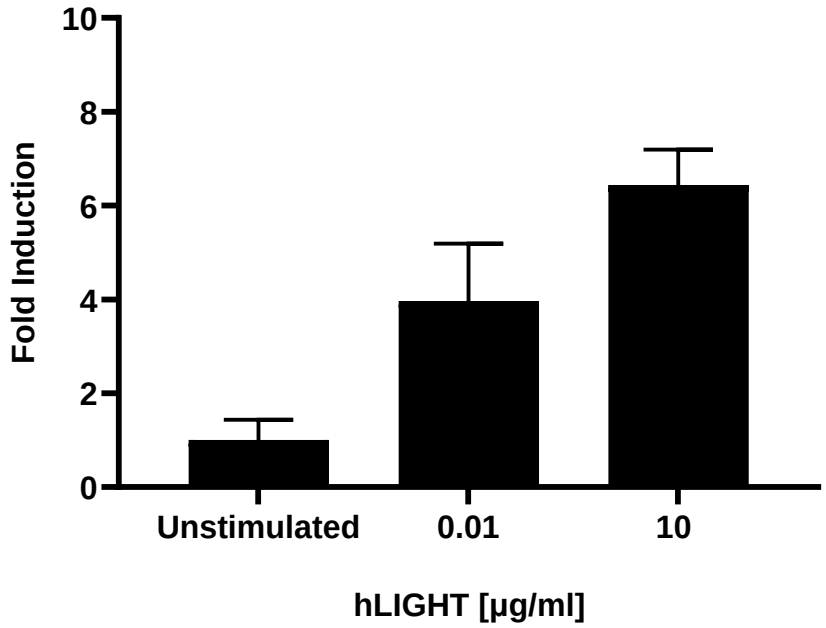

Figure 2. NF-κB Luciferase Reporter A549 Cell Line response to LIGHT.
NF-κB Luciferase Reporter A549 cells were treated with different concentrations of LIGHT. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

B. Dose Response of NF-κB Luciferase Reporter A549 Cell Line to IKK-16

1. Harvest NF-κB Luciferase Reporter cells from culture in Growth Medium 6B and seed cells at a density of 10,000 cells per well in 40 µl of Assay Medium into white opaque 96-well microplate. Leave empty wells as cell-free control wells ("Background Control").

2. Incubate at 37°C with 5% CO_2 for 6 hours.

3. Prepare a serial dilution of IKK-16 in Assay Medium at 2x the final testing concentrations (50 µl/well).

4. Add 50 µl of diluted IKK-16 to the "Test Inhibitor" wells.

5. Add 50 µl of Assay Medium to the "Unstimulated Control" wells (for measuring uninduced level of NF-κB reporter activity).

6. Add 100 µl of Assay Medium to "Background Control" wells (cell-free wells).

7. Incubate at 37°C with 5% CO_2 overnight.

8. Prepare a 10 ng/ml hTNFα solution in Assay Medium (10 µl/well).

9. Add 10 µl of hTNFα solution to the "Test Inhibitor" wells.

10. Add 10 µl of Assay Medium to the "Unstimulated Control" wells.

11. Incubate at 37°C with 5% CO_2 for 6 hours.

12. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

13. Incubate at RT for ~15 to 30 minutes.

14. Measure luminescence using a luminometer.

13. The "Background Control" luminescence value should be subtracted from all readings.

14. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

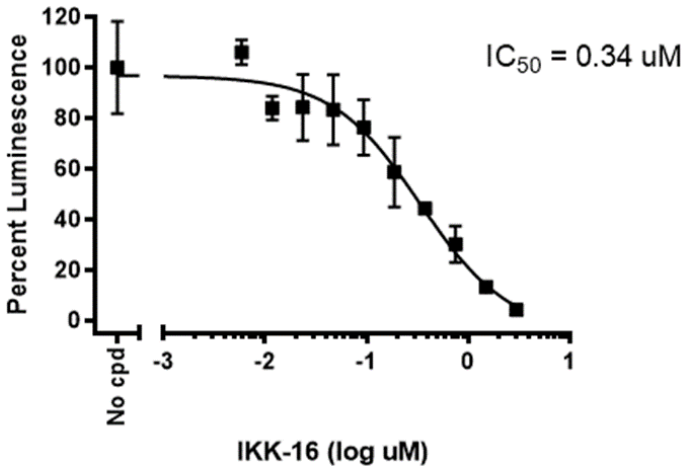

Figure 3. Dose response curve of NF-κB Luciferase Reporter A549 Cell Line to the inhibitor IKK-16. NF-κB Luciferase Reporter A549 cells were treated with increasing concentrations of IKK-16. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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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:

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Chen W., *et.al.,* 2011 *Front. Biosci.* 16: 1172-1185.
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Schmeck B., *et.al.,* 2007 *Eur. Respir. J.* 29: 25-33

Related Products

Products	*Catalog #*	*Size*
NF-κB Luciferase Reporter HCT116 Cell line	60623	2 vials
NF-κB Luciferase Reporter NIH/3T3 Cell line	79469	2 vials
NF- κB Luciferase Reporter THP-1 Cell Line	79645	2 vials
NF- κB Luciferase Reporter Raw 264.7 Cell line	79978	2 vials
NF-κB Luciferase Reporter Cell Based Assay Kit	60614	500 reactions
CD137 (4-1BB) /NF-κB Luciferase Reporter HEK293 Cell Line	79289	2 vials

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