Description

The NF-κB reporter (Luc)-THP-1 cell line is designed for monitoring NF-κB (nuclear factor 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 NOD agonists, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter gene.

Background

The role of NF-κB (nuclear factor-κB) activation is well-characterized in canonical (classical) and noncanonical (alternative) signaling pathways of inflammation. Two major forms of innate immune sensors are Toll-like receptors (TLR) and NOD/CATERPILLAR proteins. Mutations in NOD2 (nucleotide-binding oligomerization domain-containing protein 2) have been linked to chronic autoinflammatory and autoimmune diseases, such as Crohn's disease and Blaus syndrome. Studying the canonical and noncanonical NF-κB pathways and the influence of TLR pathways and NOD2 mutations can further our understanding of autoimmune regulation.

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 ~4 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience, #79796)

Host Cell

THP-1 Human leukemia monocytic cell line. Non-adherent 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 8	BPS Bioscience #79652
Growth Medium 8A	BPS Bioscience #79653

Materials Required for Cellular Assay

Name	Ordering Information
Recombinant Human hTNFα	R&D Systems #210-TA
LPS-EK (TLR4 and TLR2 agonist)	Invivogen #tlrl-eklps
L18-MDP (NOD2 agonist)	Invivogen #tlrl-lmdp
IKK-16 dihydrochloride	Sigma #SML1138
Assay Medium: Thaw Medium 8	BPS Bioscience #79652
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 the presence of the transfected gene(s) over passages.
Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 8 (BPS Bioscience, #79652):
RPMI1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin

Growth Medium 8A (BPS Bioscience, #79653):
RPMI1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin plus 1 µg/ml of Puromycin

Media Required for Functional Cellular Assay

Thaw Medium 8 (BPS Bioscience, #79652):
RPMI1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin

Cell Culture Protocol

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 8 **(no Puromycin).**

   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 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 8 **(no Puromycin).**

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

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 8 **(no Puromycin)** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 8A **(contains Puromycin)**.

Cell Passage
Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.5 x 10^6 cells/ml of Growth Medium 8A **(contains Puromycin).** The sub-cultivation ratio should maintain the cells between 0.5 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~4 x 10^6 cells/ml.

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

3. Transfer the vials to liquid nitrogen the next day for storage.

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

Note: Use Thaw Medium 8 (BPS Bioscience #79652) as assay medium.

A. Agonists dose response on NF-κB reporter (Luc) – THP-1 cells

Note: All conditions should be performed at least in triplicates. This protocol was designed for a 96-well format.

1. Seed NF-κB reporter (Luc)-THP-1 cells at a density of 25,000 cells per well into clear-bottom white 96-well plate in 50 µl of Thaw Medium 8.

2. Prepare serial dilutions of hTNFα (Tumor necrosis factor alpha), LPS (lipopolysaccharide), or L18-MDP (L18-muramyldipeptide) at concentrations 2-fold higher than the final desired concentrations. Add 50 µl of serial dilutions to the cells.

3. Add 50 µl of Thaw Medium 8 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 for 5-6 hours.

6. After incubating for ~5 to 6 hours, perform luciferase assay using the **ONE-Step™ Luciferase Assay System**. Add 100 µl of **ONE-Step™** Luciferase reagent per well and rock gently at room temperature for ~15 minutes. Measure luminescence using a luminometer.

   Note: *If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.*

7. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB luciferase reporter expression is the background-subtracted luminescence of treated cells divided by the average background-subtracted luminescence of untreated control cells.

$$Fold\ induction = \frac{average\ Lum\ of\ treated\ cells - average\ background}{average\ Lum\ of\ control\ cells - average\ background}$$

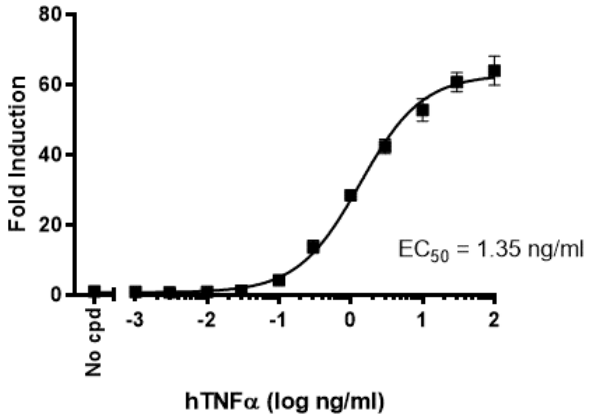

Figure 1. hTNFα dose response in NF-κB reporter (Luc)-THP-1 cells. Cells were incubated with increasing concentrations of recombinant human TNFα (R&D Systems, #210-TA) for 5-6 hours before measuring luciferase activity using ONE-Step™ Luciferase Assay System.

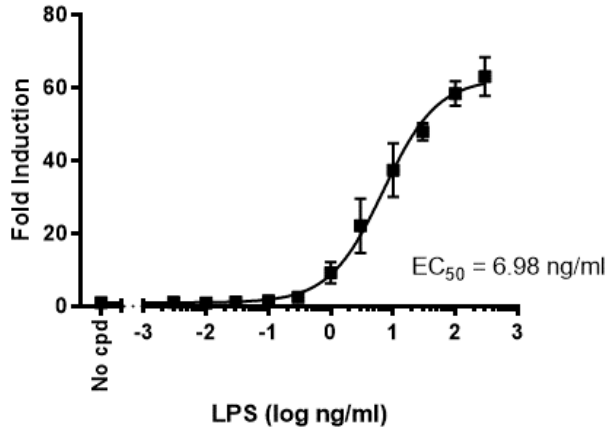

Figure 2. LPS dose response in NF-κB reporter (Luc)-THP-1 cells. Cells were incubated with increasing concentrations of TLR4 and TLR2 agonist LPS-EK (lipopolysaccharide, Invivogen, #tlrl-eklps) for 5-6 hours before measuring luciferase activity using ONE-Step™ Luciferase Assay System.

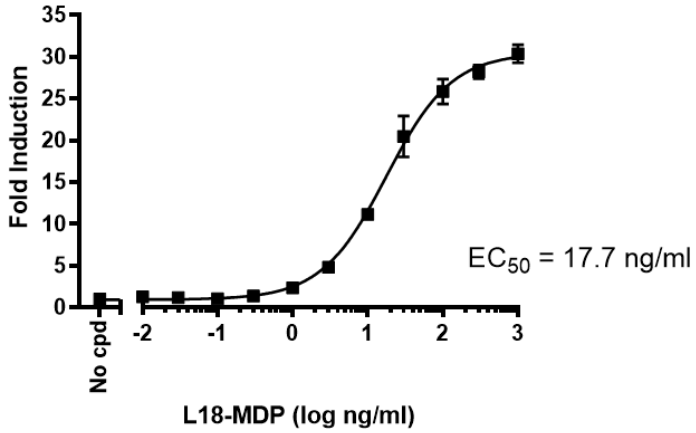

Figure 3. L18-MDP dose response in NF-κB reporter (Luc)-THP-1 cells. Cells were incubated with increasing concentrations of NOD2 agonist L18-MDP (L18-muramyldipeptide, Invivogen, tlrl-lmdp) for 5-6 hours before measuring luciferase activity using ONE-Step™ Luciferase Assay System.

B. Testing inhibitors on NF-κB reporter (Luc) – THP-1 cells

Cells *must* be stimulated with an agonist while testing inhibitors. Unstimulated cells will display very low luciferase activity. In the following experiments, cells were treated with agonists hTNFα or LPS following treatment with IKK-16 inhibitor.

Note: All conditions should be performed at least in triplicates. This protocol was designed for a 96-well format.

1. Seed NF-κB reporter (Luc)-THP-1 cells at a density of 25,000 cells per well into clear-bottom, white 96-well plate in 40 µl of Thaw Medium 8.

2. Prepare serial dilutions of test compounds or IKK-16 control at concentrations 2-fold higher than the final desired concentrations in Thaw Medium 8. Add 50 µl of dilutions to cells.

3. Add 50 µl of Thaw Medium 8 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 or LPS agonists at concentrations 10-fold higher than the desired final concentrations in Thaw Medium 8. Final concentration added to the cells: TNFα = 10 ng/ml, LPS = 100 ng/ml. Add 10 µl of diluted hTNFα or LPS to the wells with test inhibitors.

7. Add 10 µl of Thaw Medium 8 to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).

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

8. After incubating for ~5 to 6 hours, perform the luciferase assay using the **ONE-Step™ Luciferase Assay System**. Add 100 µl of **ONE-Step™** Luciferase reagent per well and rock gently at room temperature for ~15 minutes. Measure luminescence using a luminometer.

   Note: If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

9. Data Analysis: Subtract the average background luminescence (cell-free control wells) from luminescence readings of all wells. Results are expressed as percent of control (stimulated cells without inhibitor), set to 100%. The percent luminescence of NF-κB luciferase reporter activity is the (background-subtracted luminescence of IKK-16 inhibitor-treated cells divided by the background-subtracted luminescence of untreated control wells) multiplied by 100%.

$$\%\ Lum = \left(\frac{Lum\ of\ inhibitor\ treated\ cells - background}{Lum\ of\ control\ wells - background}\right) x100$$

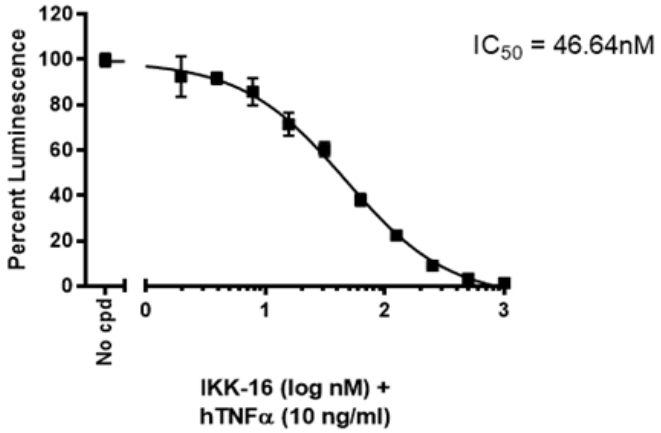

Figure 4. IKK-16 inhibition of hTNFα stimulation in NF-κB reporter (Luc)-THP-1 cells. Increasing concentrations of IKK-16, an inhibitor of IκB IKK, were incubated with NF-κB reporter (Luc)-THP-1 cells overnight. Cells were stimulated with 10 ng/ml of recombinant human TNFα (R&D Systems, #210-TA) for 5-6 hours before measuring luciferase activity using ONE-Step™ Luciferase Assay System.

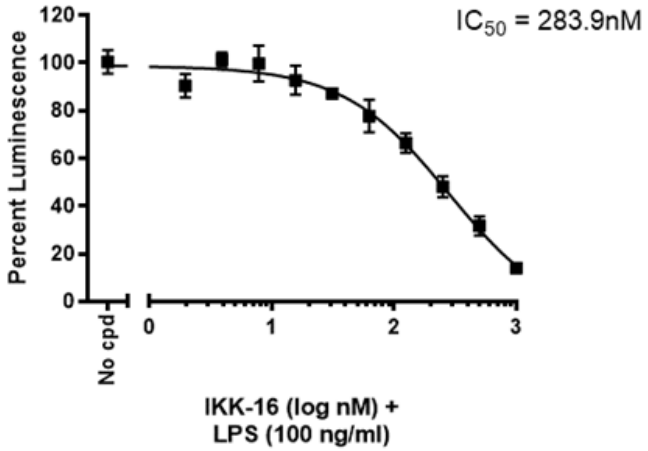

Figure 5. IKK-16 inhibition of LPS stimulation in NF-κB reporter (Luc)-THP-1 cells.
Increasing concentrations of inhibitor IKK-16 were incubated with NF-κB reporter (Luc)-THP-1 cells overnight. Cells were stimulated with 100 ng/ml of TLR4 and TLR2 agonist LPS-EK (lipopolysaccharide, Invivogen, #tlrl-eklps) for 5-6 hours before measuring luciferase activity using ONE-Step™ Luciferase Assay System.

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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.
3. Pan, Q., Kravchenko, V., Katz, A., Huang, S., Ii, M., Mathison, J. C., Kobayashi, K., Flavell, R. A., Schreiber, R. D., Goeddel, D., & Ulevitch, R. J. (2006). NF-κB-Inducing Kinase Regulates Selected Gene Expression in the Nod2 Signaling Pathway. *Infection and Immunity*, *74*(4), 2121–2127.

Related Products

Products	*Catalog #*	*Size*
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB Reporter (Luc) - A549 Cell Line	60625	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) - Jurkat Cell Line	60651	2 vials
ONE-Step™ Luciferase Assay System	60690	Multiple sizes
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 reactions