Description

NF-κB reporter (Luc)-THP-1 Cell Line is a THP-1 cell line designed for monitoring NF-κB (nuclear factor κB) signal transduction pathways. It contains a firefly luciferase reporter 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.

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/CATERPILLER 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 >1 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

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Materials Required for Cellular Assay

Name	Ordering Information
Recombinant Human hTNFα Protein	R&D Systems #210-TA
LPS-EK (TLR4 and TLR2 agonist)	Invivogen #tlrl-eklps
L18-MDP (NOD2 agonist)	Invivogen #tlrl-lmdp
Recombinant Human IL-1 beta/IL-1F2 Protein	R&D Systems #201-LB
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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. 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 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 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.

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Cell Culture Protocol

Note: THP-1 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 8.

   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 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 8.

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 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.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.5 x 10^6 cells/ml in Growth Medium 8A. 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 Cell Freezing Medium (BPS Bioscience #79796) at a density of ~4 x 10^6 cells/ml.

2. 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.

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

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.
- Assay A should include “Stimulated”, “Background Control” and “Unstimulated Control” conditions.
- Assay B should include “Background Control”, “No Inhibitor Control”, “No Inhibitor, No Agonist Control” and “Test Inhibitor” conditions.

- NF-κB reporter (Luc)-THP-1 cells were not responsive to hIL-6 (R&D Systems #206-IL), hIL-8 (R&D Systems #208-IL) or hIFNγ (R&D Systems #285-IF) at up to 100 ng/ml, or hIL-18/IL-1F4 (R&D Systems #9124-IL) at up to 1,000 ng/ml.

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

A. Agonists dose response on NF-κB Reporter (Luc) – THP-1 Cell Line

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. Leave empty wells as cell-free control wells (“Background Control”).

2. Prepare serial dilutions of hTNFα (Tumor necrosis factor alpha), LPS (lipopolysaccharide), L18-MDP (L18-muramyldipeptide) or hIL-1b at concentrations 2-fold higher than the final desired concentrations in Assay Medium (50 µl/well).

3. Add 50 µl of the serial dilutions to the “Stimulated” wells.

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

5. Add 100 µl of Assay Medium to “Background Control”.

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

7. Incubate for ~5 to 6 hours.

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

9. Rock gently at Room Temperature (RT) for ~15 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 (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 (“Stimulated”) divided by the average background-subtracted luminescence of untreated control cells (“Unstimulated Control”).

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

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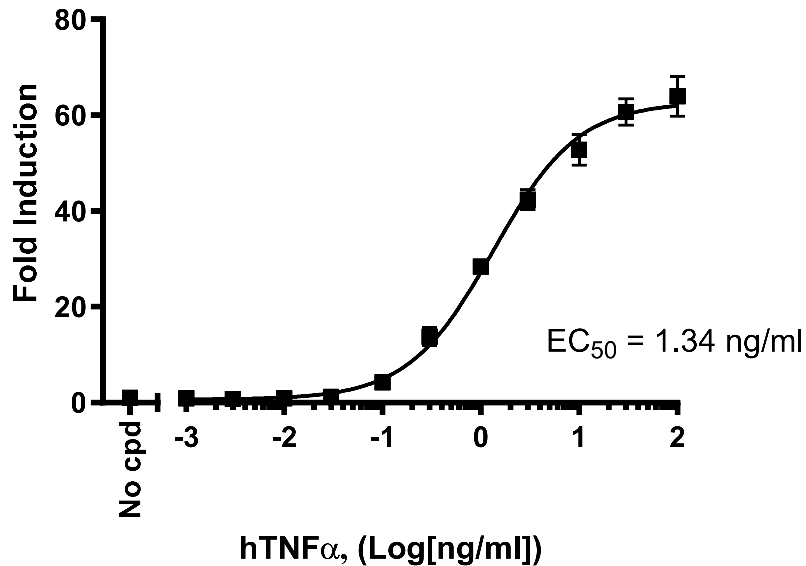

Figure 1. Dose response curve of NF-κB Reporter (Luc)-THP-1 Cell Line to hTNFα.
NF-κB Reporter (Luc) - THP-1 cells were incubated with increasing concentrations of recombinant human TNFα (R&D Systems #210-TA) for 5-6 hours before measuring luciferase activity with ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of unstimulated.

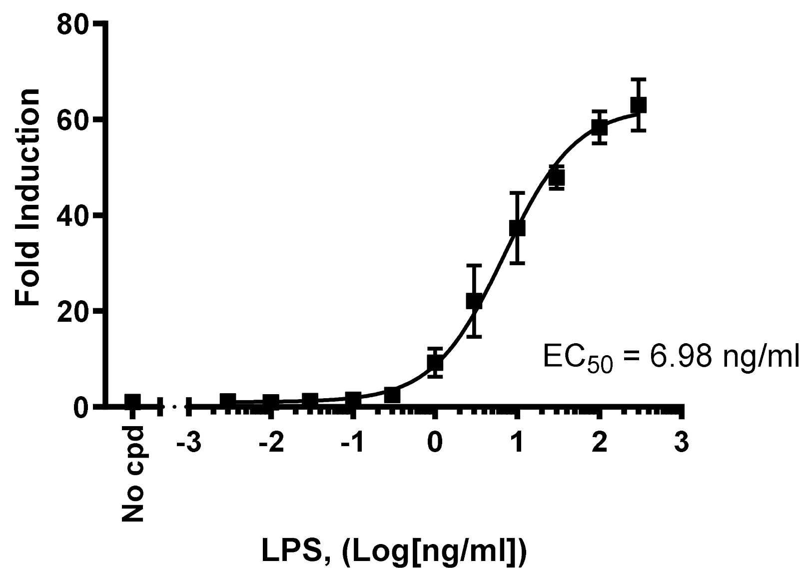

Figure 2. Dose response curve of NF-κB Reporter (Luc)-THP-1 Cell Line to LPS.
NF-κB Reporter (Luc)-THP-1 cells were incubated with increasing concentrations of the TLR4 and TLR2 agonist LPS-EK (Invivogen #tlrl-eklps) for 5-6 hours before measuring luciferase activity with ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of unstimulated.

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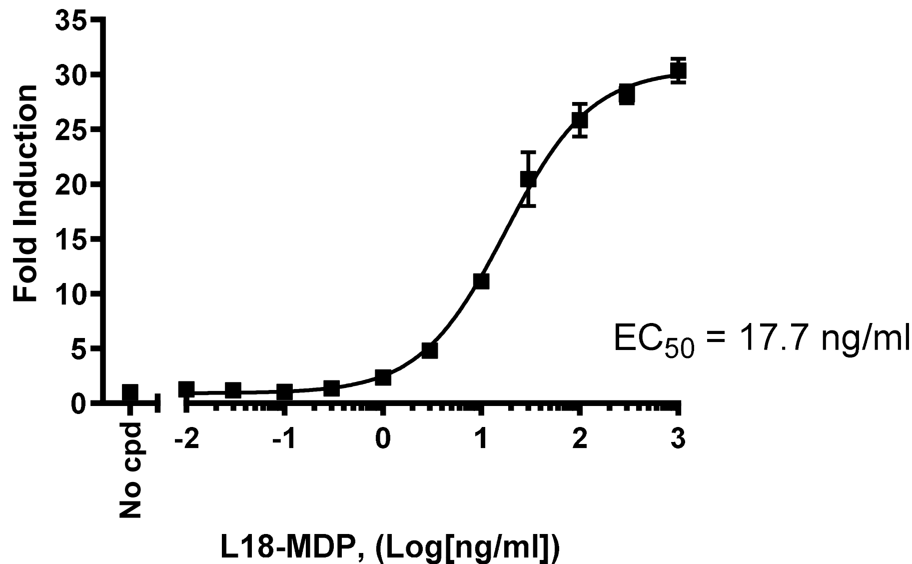

Figure 3. Dose response curve of NF-κB reporter (Luc)-THP-1 Cell Line to L18-MDP.
NF-κB Reporter (Luc)-THP-1 cells were incubated with increasing concentrations of the NOD2 agonist L18-MDP (L18-muramyldipeptide) (Invivogen #tlrl-lmdp) for 5-6 hours before measuring luciferase activity with ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of unstimulated.

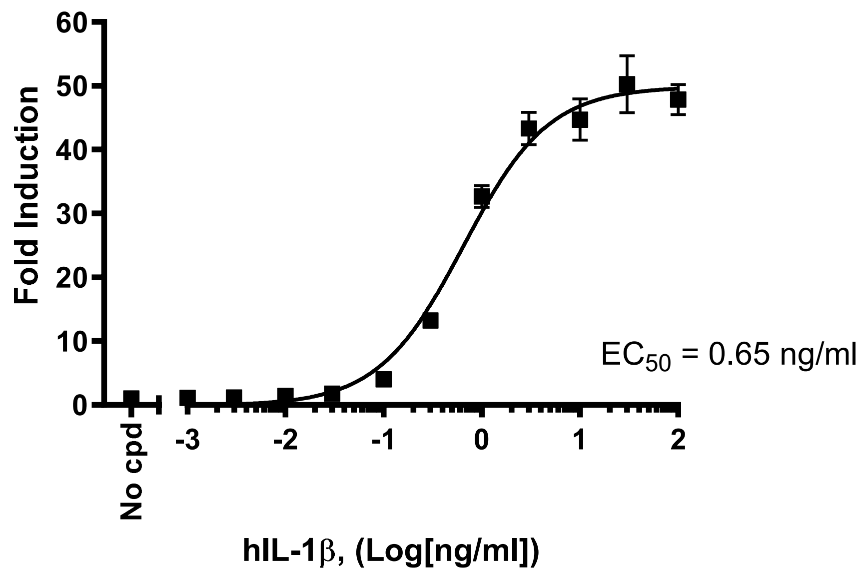

Figure 4. Dose response curve of NF-κB reporter (Luc)-THP-1 Cell Line to hIL-1b.
NF-κB Reporter (Luc)-THP-1 cells were incubated with increasing concentrations of hIL-1b (R&D Systems #201-LB) for 5-6 hours before measuring luciferase activity with ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of unstimulated.

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B. Inhibition of induced reporter activity in the NF-κB Reporter (Luc) – THP-1 Cell Line

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 the agonists hTNFα, LPS, or hIL-1b following treatment with the IKK-16 inhibitor.

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. Leave a few wells empty to use as the "Background Control" (cell-free control).

2. Prepare serial dilutions of test compounds or IKK-16 at concentrations 2-fold higher than the final desired concentrations in Assay Medium (50 µl/well).

3. Add 50 µl of diluted inhibitor to the "Test Inhibitor" wells.

4. Add 50 µl of Assay Medium to the "No Inhibitor Control" and "No Inhibitor, No Agonist Control" wells.

5. Add 110 µl of Assay Medium to the "Background Control" wells.

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

7. Prepare hTNFα, LPS or hIL-1b agonists at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

   Note: The recommended final concentration added to the cells is: TNFα = 10 ng/ml, LPS = 100 ng/ml.

8. Add 10 µl of diluted hTNFα, LPS or hIL-1b to the "Test Inhibitor" and "No Inhibitor Control" wells.

9. Add 10 µl of Assay Medium to the "No Inhibitor, No Agonist Control" wells.

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

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

12. Rock gently at room temperature for ~15 minutes.

13. Measure luminescence using a luminometer.

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

15. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of luciferase reporter expression is the background-subtracted luminescence of treated wells ("Test Inhibitor") divided by the average background-subtracted luminescence of the untreated control wells ("No Inhibitor Control") x 100%.

$$Percent\ Luminescence = \left(\frac{Luminescence\ of\ \ Test\ Inhibitor\ Wells - avg.background}{Avg.Luminescence\ of\ No\ Inhibitor\ Wells - avg.background}\right)x100$$

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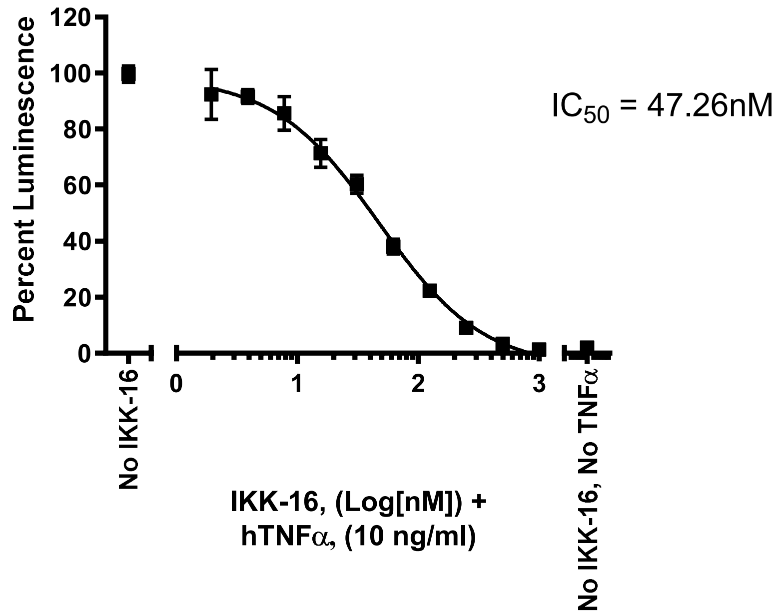

Figure 5. IKK-16 inhibition of the hTNFα induced stimulation in NF-κB reporter (Luc)-THP-1 Cell Line.

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 with ONE-Step™ Luciferase Assay System.

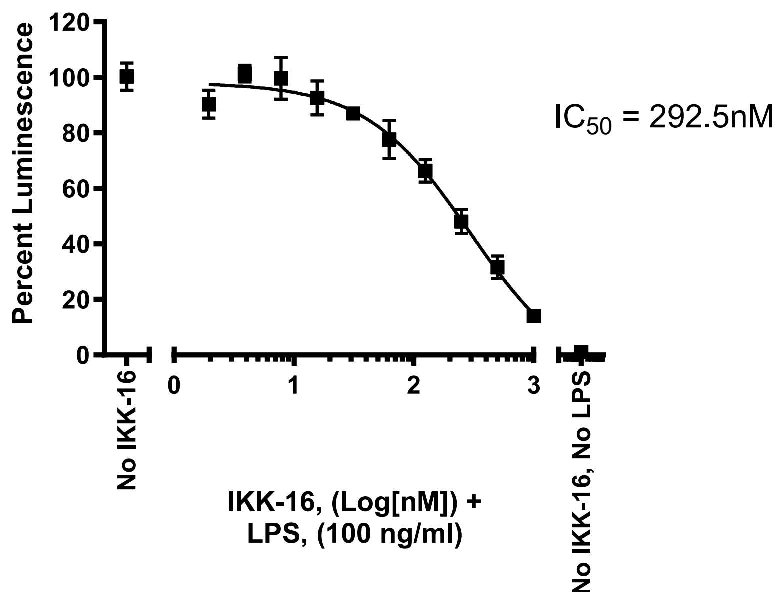

Figure 6. IKK-16 inhibition of the LPS induced stimulation in NF-κB reporter (Luc)-THP-1 Cell Line. Increasing concentrations of inhibitor IKK-16 were incubated with NF-κB reporter (Luc)-THP-1 cells overnight. Cells were stimulated with 100 ng/ml of the TLR4 and TLR2 agonist LPS-EK (Invivogen, #tlrl-eklps)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

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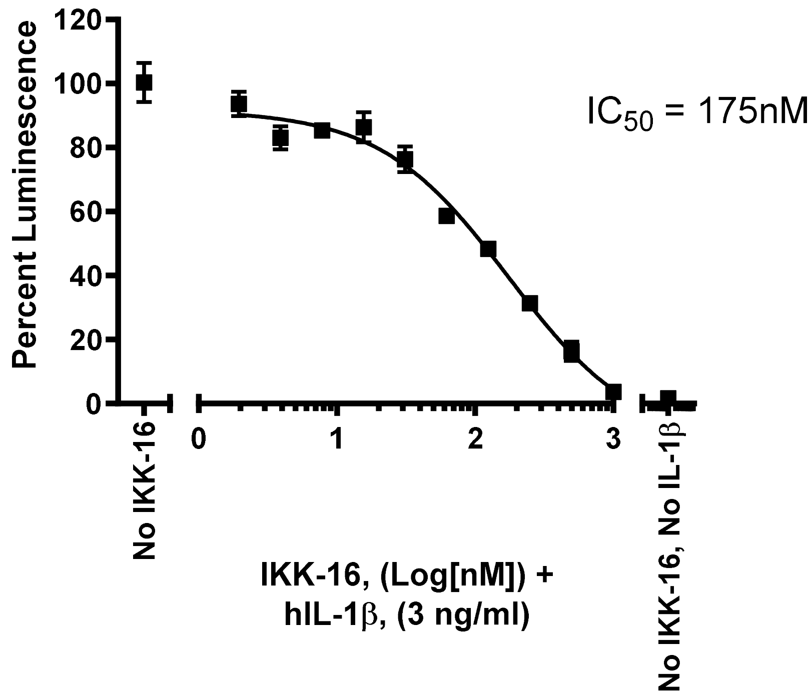

Figure 7. IKK-16 inhibition of the hIL-1b induced stimulation in NF-κB reporter (Luc)-THP-1 Cell Line. Increasing concentrations of IKK-16, an inhibitor of IκB IKK, were incubated with NF-κB reporter (Luc)-THP-1 cells overnight. Cells were stimulated with 3 ng/ml of recombinant human IL-1b (R&D Systems #201-LB) for 5-6 hours before measuring luciferase activity with ONE-Step™ Luciferase Assay System.

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

Pessara U. and Koch N., 1990 *Mol Cell Biol.* 10(8):4146-4154.
Baeuerle P.A., 1998 *Curr Biol.* 8(1):R19-R22.
Pan, Q., *et al.*, 2006 *Infection and Immunity*, *74*(4), 2121–2127.

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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
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 reactions

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