Description

NF-κB Reporter (Luc) HEK293 Cell Line is a HEK293 cell designed to monitor nuclear factor Kappa B (NF-κB) activity. 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 agonists of lymphokine receptors, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter. The cell line has been functionally validated in response to human TNF-α, IL-1β, and IL-17.

Application

- Monitor NF-κB 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

HEK293, epithelial-like cells, adherent

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 1	BPS Bioscience #60187
Growth Medium 1C	BPS Bioscience #79532

Materials Required for Cellular Assays

Name	Ordering Information
Recombinant Human TNF-alpha Protein (hTNFα)	R&D Systems 210-TA
Human Interleukin-1 beta Recombinant (IL-1β)	BPS Bioscience #90168
hIL-17A, Avi-Tag Recombinant	BPS Bioscience #91014
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
IKK-16 dihydrochloride (inhibitor of NF-κB activation)	Sigma #SML1138
Brodalumab (Anti-IL-17R Antibody)	MedChemExpress #HY-P9925
Anti-IL-17A Neutralizing Antibody	BPS Bioscience #91015
96-well tissue culture-treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

Storage Conditions

Cells will arrive in 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 Bioscience's 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℃ 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 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1C (BPS Bioscience #79532):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin and 50 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium:

Thaw Medium 1 (BPS Bioscience #60187)

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

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.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Transfer the contents of the cryovial to a tube containing 10 ml of Thaw Medium 1.

3. 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 1.

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

5. After 24 hours of culture, check for cell viability and attachment. For a T25 flask, add 3-4 ml of Thaw Medium 1, and continue growing in a 5% CO2 incubator at 37°C until the cells are ready to passage.

6. Cells should be passaged before they reach full confluency. Switch to Growth Medium 1C at first and subsequent passages.

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 1C 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 1C.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10-1:20 weekly.

Cell Freezing

1. Aspirate the medium, wash the cells with 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 1C and count the cells.

3. 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.

4. 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.

5. 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.
- 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.

A. Dose Response of NF-κB Reporter (Luc) HEK293 Cell Line to Agonists

1. Seed NF-κB Reporter (Luc) HEK293 cells at a density of 30,000 cells per well into a white, clear-bottom 96-well culture plate in 75 µl of Assay Medium. Leave empty wells as cell-free control wells (“Background Control”).

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

3. Prepare a threefold serial dilution of agonist in Assay Medium at concentrations 4-fold higher than the final desired concentrations (25 µl/well).

4. Add 25 µl of each dilution to the wells labeled as "Stimulated".

5. Add 25 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.

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. Rock at room temperature for ~15 minutes.

10. Measure luminescence using a luminometer.

11. Subtract the background luminescence value from all measurements.

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 stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

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

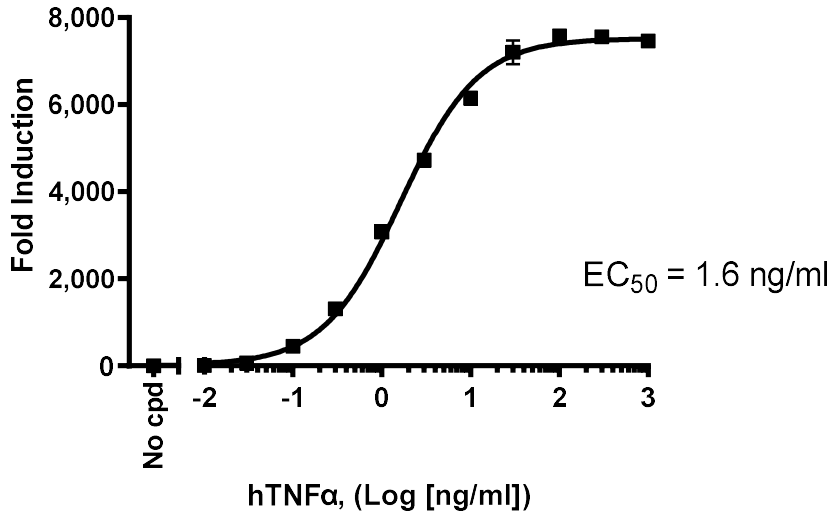

Figure 1: Dose response curve of NF-κB Reporter (Luc) HEK293 Cell Line to hTNFα.
NF-κB Reporter (Luc) HEK293 cells were treated with increasing doses of hTNFα for 5 hours and luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are shown as fold induction of luciferase reporter expression.

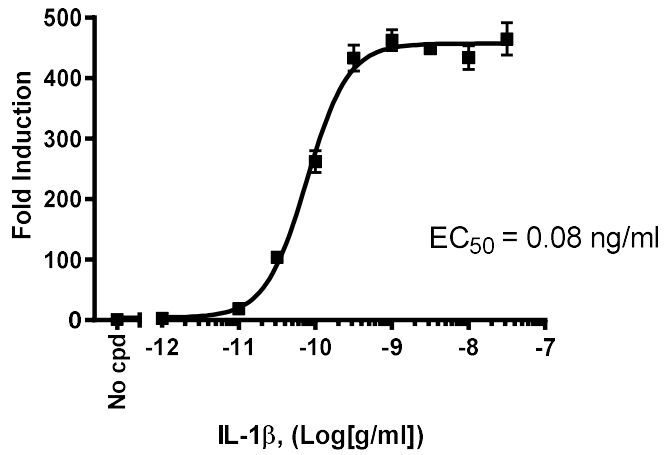

Figure 2: Dose response of NF-κB Reporter (Luc) HEK293 Cell Line to IL-1β.
NF-κB Reporter (Luc) HEK293 cells were treated with increasing doses of IL-1β for 5 hours and luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are shown as fold induction of luciferase reporter expression.

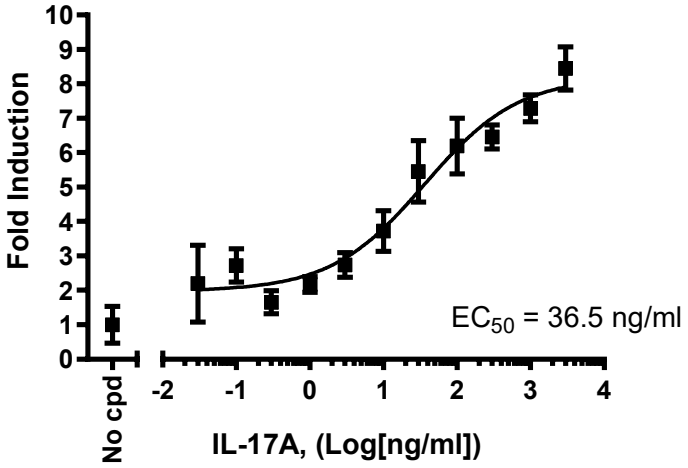

Figure 3: Dose response curve of NF-κB reporter (Luc) HEK293 Cell Line to IL-17A.
NF-κB Reporter (Luc) HEK293 cells were treated with increasing doses of IL-17A for 5 hours and luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are shown as fold induction of luciferase reporter expression.

B. Inhibition of agonist-induced NF-κB activity in NF-κB Reporter (Luc) HEK293 Cell Line

1. Seed NF-κB Reporter (Luc) HEK293 cells at a density of 30,000 cells per well into a white, clear-bottom 96-well culture plate in 50 µl of Assay Medium and allow to attach for 4-5 hours. Leave empty wells as cell-free control wells ("Background Control").

2. Prepare a serial dilution of NF-κB inhibitor in Assay Medium at concentrations two-fold higher than the final desired concentrations (50 µl/ well).

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

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

5. Incubate the cells at 37°C with 5% CO_2 overnight.

   Note: As an alternative, the inhibitor can be added to the cells next day and incubated at 37°C with 5% CO_2 for 2-4 hours before addition of agonist (see Figure 8).

6. The next day, prepare solution of agonist in Assay Medium at a concentration 11-fold higher than the final desired concentration (10 µl/well).

7. Add 10 µl of agonist solution to the wells labeled as "Test Inhibitor" and "No Inhibitor Control".

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

9. Add 110 µl of Assay Medium to the "Background 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 at room temperature for ~15 minutes.

13. Measure luminescence using a luminometer.

14. Subtract the background luminescence value from all measurements.

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 divided by the average background-subtracted luminescence of the untreated control wells x 100%.

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

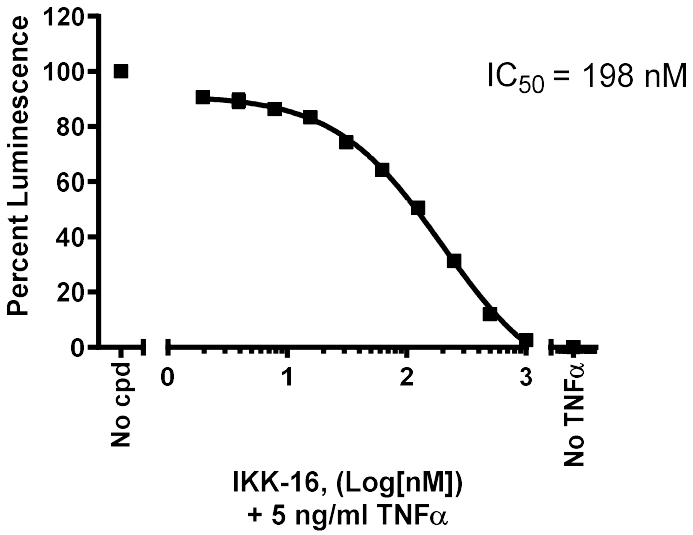

Figure 4: Inhibition of TNFα-induced NF-κB activity by the NF-κB inhibitor IKK-16 dihydrochloride in the NF-κB Reporter (Luc) HEK293 Cell Line.
NF-κB Reporter (Luc) HEK293 cells were pre-incubated with increasing doses of IKK-16 prior to stimulation with 5 ng/ml of hTNFα, as described in the protocol above. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence compared to wells without IKK-16 (set at 100%).

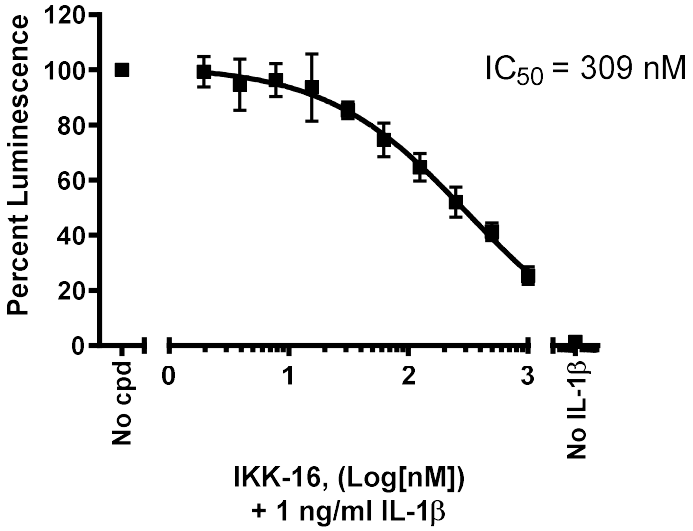

Figure 5: Inhibition of IL-1β-induced NF-κB activity by the NF-κB inhibitor IKK-16 dihydrochloride in the NF-κB Reporter (Luc) HEK293 Cell Line.
NF-κB Reporter (Luc) HEK293 cells were pre-incubated with increasing doses of IKK-16 prior to stimulation with 1 ng/ml of IL-1b, as described in the protocol above. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence compared to wells without IKK-16 (set at 100%).

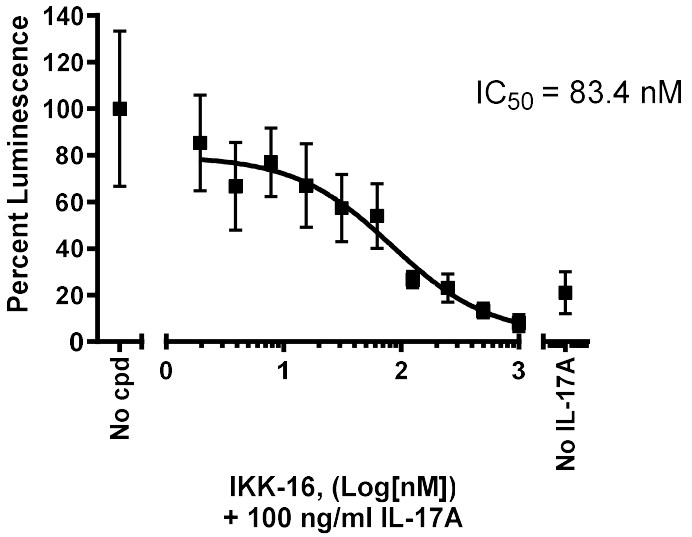

Figure 6: Inhibition of IL-17A-induced NF-κB activity by the NF-κB inhibitor IKK-16 dihydrochloride in the NF-κB Reporter (Luc) HEK293 Cell Line.
NF-κB Reporter (Luc) HEK293 cells were pre-incubated with increasing doses of IKK-16 prior to stimulation with 100 ng/ml of IL-17A, as described in the protocol above. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence compared to wells without IKK-16 (set at 100%).

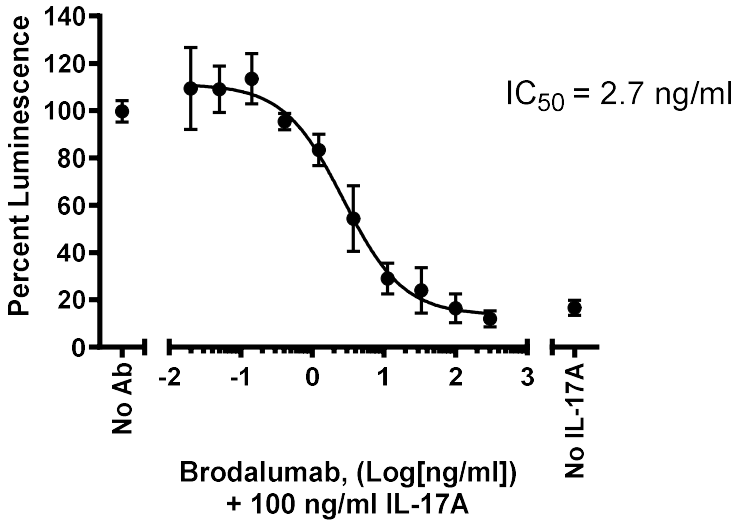

Figure 7: Inhibition of IL-17A-induced NF-κB activity by Brodalumab, an anti-IL-17-receptor antibody, in the NF-κB Reporter (Luc) HEK293 Cell Line.
NF-κB Reporter (Luc) HEK293 cells were pre-incubated with increasing doses of Brodalumab prior to stimulation with 100 ng/ml of IL-17A, as described in the protocol above. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence compared to wells without antibody (set at 100%).

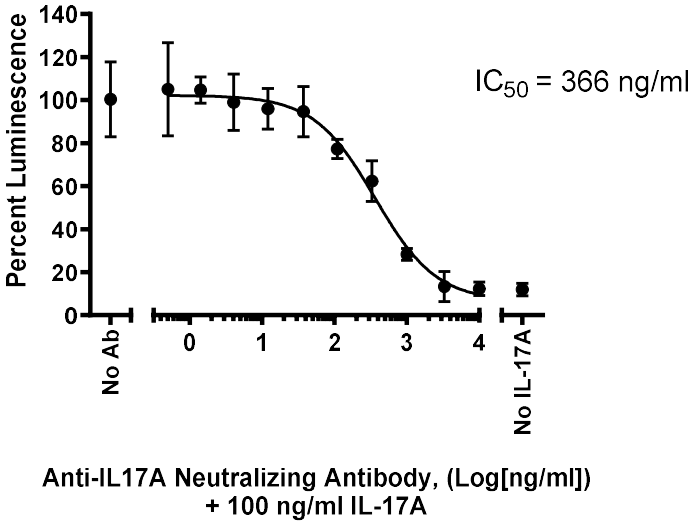

Figure 8: Inhibition of IL-17A-induced NF-κB activity by Anti-IL-17 Neutralizing Antibody in the NF-κB Reporter (Luc) HEK293 Cell Line.
NF-κB Reporter (Luc) HEK293 cells were pre-incubated with increasing doses of anti-IL-17A Neutralizing Antibody for 1 hour, followed by a 5–6-hour stimulation period with 100 ng/ml of human IL-17A. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence compared to wells without antibody (set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at 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.
Takada Y., *et al*., 2005 *J Biol Chem.* 280(17): 17203-17212.

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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.

Related Products

Products	*Catalog #*	*Size*
NF- κB Reporter (Luc) – THP-1 Cell Line	79645	2 vials
NF- κB Reporter (Luc) – NIH/3T3 Cell Line	79469	2 vials
CD27/NF-κB Reporter-Jurkat Cell Line	79509	2 vials
TLR8/NF-κB Reporter-HEK293 Cell Line	60684	2 vials
GITR/NF-κB Reporter-Jurkat Cell Line	60546	2 vials

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