Description

The NF-κB reporter (Luc) HEK293 cell line is designed to monitor nuclear factor Kappa B (NF-κB) activity. 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 agonists of lymphokine receptors, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter gene. 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 2 x 10^6 cells in 1 ml of 90% FBS, 10% DMSO

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
hTNFα	R&D Systems 210-TA
IL-1β	BPS Bioscience #90168
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1C	BPS Bioscience #79532
IKK-16 dihydrochloride: inhibitor of NF-κB activation	Sigma #SML1138
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	

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 for maintaining cell lines over many passages. Cells should be grown at 37°C 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 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

Assay Medium: Thaw Medium 1

Cell Culture Protocol

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, transfer to a tube containing 10 ml of Thaw Medium 1 (**no hygromycin**), spin down cells, resuspend cells in pre-warmed Thaw Medium 1 (**no hygromycin**), transfer resuspended cells to T25 flask and culture in 37°C CO_2 incubator.

2. At first passage switch to Growth Medium 1C (**contains hygromycin**). Cells should be split before they reach complete confluence.

Cell Passage

1. To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA, add Growth Medium 1C (**contains hygromycin**) and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:10 to 1:20 weekly.

Cell Freezing

1. To cryopreserve the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.

2. After detachment, add Growth Medium 1C and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience #79796 or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.

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

4. Transfer to liquid nitrogen the next day for 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 and Assay Performance

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

A. Agonist dose response

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

2. Prepare threefold serial dilutions of agonist in assay medium at concentrations 4-fold higher than the final desired concentrations and add 25 µl of each dilution to wells labeled as "stimulated".

3. Add 25 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 for 5-6 hours.

6. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.

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

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells - background}{luminescence\ of\ unstimulated\ wells - background}$$

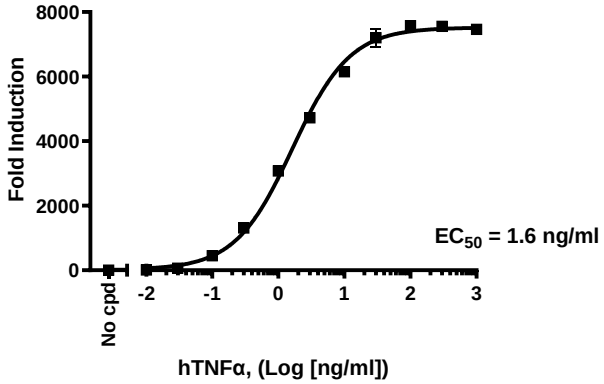

Figure 1: Dose response of hTNFα in NF-κB reporter (Luc) HEK293 cells. The cells were treated with increasing doses of TNFα for 5 hours and luciferase activity was measured using the ONE-Step™ luciferase assay system. Results are shown as fold induction of luciferase reporter expression.

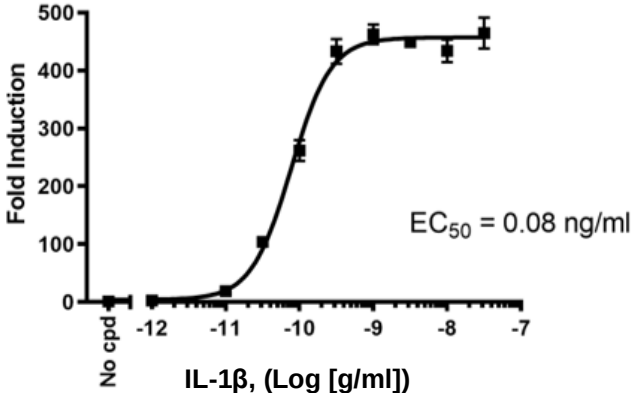

Figure 2: Dose response of IL-1β in NF-κB reporter (Luc) HEK293 cells. The cells were treated with increasing doses of IL-1β for 5 hours and luciferase activity was measured using the ONE-Step™ luciferase assay system. Results are shown as fold induction of luciferase reporter expression.

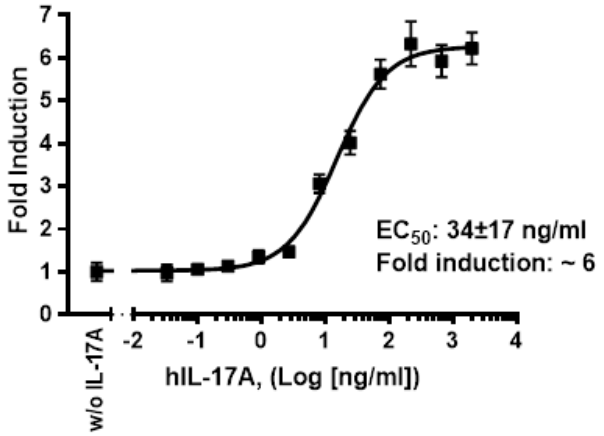

Figure 3: Dose response of hIL-17 in NF-κB reporter (Luc) HEK293 cells. The cells were treated with increasing doses of IL-17 for 5 hours and luciferase activity was measured using the ONE-Step™ luciferase assay system. Results are shown as fold induction of luciferase reporter expression.

B. Inhibition of agonist-induced NF-κB activity

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.

2. Add 50 µl of assay medium with or without NF-κB inhibitor serial dilutions to wells.

3. Incubate the cells at 37°C with 5% CO_2 overnight. As an alternative, the inhibitor can be added to cells the next day and incubated at 37°C with 5% CO_2 for 2-4 hours before addition of the agonist.

4. Add the agonist of interest in 10 µl of assay medium to the wells labeled as "stimulated".

5. Add 10 µl of assay medium to the unstimulated control wells.

6. Add 110 µl of assay medium to cell-free control wells (to determine the background signal).

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

8. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer. Subtract the background luminescence value from all measurements.

9. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence of NF-κB luciferase reporter expression is the average background-subtracted luminescence of inhibitor-treated wells divided by the average background-subtracted luminescence of untreated wells, reported to the inhibitor-treated wells set at 100%.

$$\%\ Luminescence = \left(\frac{luminescence\ of\ inhibitor\ treated\ wells - background}{luminescence\ of\ untreated\ wells - background}\right) x100$$

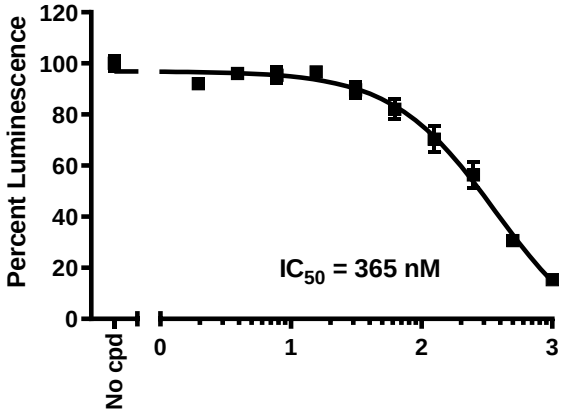

Figure 4: Inhibition of TNFα-induced NF-κB activity by NF-κB inhibitor IKK-16 dihydrochloride, in NF-κB reporter (Luc) HEK293 cells. Cells were pre-incubated with increasing doses of IKK-16 prior to stimulation with 5 ng/ml hTNFα, as described in the protocol above. The results are shown as percent luminescence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

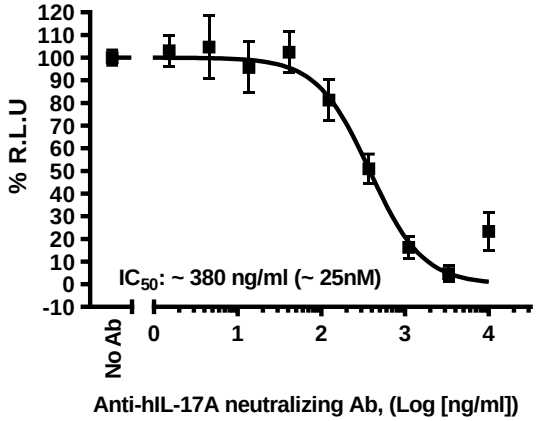

Figure 5: Inhibition of IL-17-induced NF-κB activity by anti-IL-17 neutralizing antibody, in NF-κB reporter (Luc) HEK293 cells. Cells were pre-incubated with increasing doses of the antibody for 1 hour before stimulation with 100 ng/ml human IL-17 for 5-6 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay system. The results are shown as percent luminescence compared to wells without antibody (set at 100%).

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. Takada Y, Kobayashi Y, Aggarwal BB (2005) Evodiamine abolishes constitutive and inducible NF-κB activation by inhibiting IκBα kinase activation, thereby suppressing NF-κB-regulated antiapoptotic and metastatic gene expression, up-regulating apoptosis, and inhibiting invasion. *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*
IL-1β	90168	2 µg/10 µg
Thaw Medium 1	60187	100 ml
Growth Medium 1C	79532	500 ml
NF- κB Reporter (Luc) – THP-1 Cell Line	79645	2 vials
NF- κB Reporter (Luc) – NIH/3T3 Cell Line	79469	2 vials
NF- κB Reporter (Luc) – Jurkat Cell Line	60651	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 Kit	60614	500 reactions
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