Description

The NF-κB Luciferase Reporter Jurkat Cell Line is a Jurkat cell line designed for monitoring nuclear factor Kappa B (NF-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 activators of lymphokine receptors, endogenous NF-κB transcription factor binds to the DNA response elements, inducing transcription of the luciferase reporter.

This cell line has been functionally validated and responds to phorbol 12-myristate 13-acetate (PMA) with ionomycin and TNFα. It can be used to measure TCR (T Cell Receptor)-mediated T cell activation through co-stimulation with anti-CD3 and anti-CD28 antibodies.

Background

Nuclear factor-Kappa B (NF-κB)/Rel proteins include NF-κB2 p52/p100, NF-κB1 p50/p105, c-Rel, RelA/p65, and RelB. These proteins function as dimeric transcription factors that control genes regulating a broad range of biological processes including innate and adaptive immunity, inflammation, stress responses, B cell development, and lymphoid organogenesis. In the classical (or canonical) pathway, NF-κB/Rel proteins are bound and inhibited by IκB proteins. Proinflammatory cytokines, growth factors, and antigen receptors activate an IKK (inhibitor of κB kinase) complex (IKKβ, IKKα, and NEMO (NF-κB essential modulator)), which phosphorylates IκB proteins. Phosphorylation of IκB leads to its ubiquitination and proteasomal degradation, freeing NF-κB/Rel complexes. Free NF-κB/Rel complexes are further activated by phosphorylation and translocated to the nucleus where they induce the expression of target genes. In the alternative (noncanonical) NF-κB pathway, NF-κB2 p100/RelB complexes are inactive in the cytoplasm. Signaling through a subset of receptors including LTβR (lymphotoxin beta receptor), CD40, and BR3 (Blys receptor 3), activates the kinase NIK (NF-κB-inducing kinase), which in turn activates IKKα complexes that phosphorylate C-terminal residues in NF-κB2 p100. Phosphorylation of NF-κB2 p100 leads to its ubiquitination and proteasomal processing to NF-κB2 p52, creating transcriptionally competent NF-κB p52/RelB complexes that translocate to the nucleus and induce target gene expression. An understanding of the NF-κB pathway and how to modulate is critical to understand gene regulation in health and disease.

Applications

- Determine compound activity on the NF-κB pathway in a cellular model.
- Monitor NF-κB signaling pathway activity.
- Determine T cell receptors mediated T cell activation.

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

Jurkat (clone E6-1), human T lymphoblast, suspension

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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2B	BPS Bioscience #79530

Materials Required for Cellular Assays

Name	Ordering Information
Ionomycin	Sigma #I3909
Phorbol 12-myristate 13-acetate (PMA)	LC Laboratories #P1680
TNFα	Sigma #T0157-10UG
IKK-16 dihydrochloride: inhibitor of NF-κB activation	Sigma #SML1138
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
Anti-CD28 Agonist Antibody	BPS Bioscience #100182
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White, clear-bottom cell culture plate, 96-well	
Luminometer	

Storage Conditions

Cells are shipped 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*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* 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 2 (BPS Bioscience #60184):

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2B (BPS Bioscience #79530):

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

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

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 2 to the conical tube containing the cells. Thaw Medium 2 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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 2.

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

6. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. 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 2B.

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 0.2 x 10^6 cells/ml with Growth Medium 2B. The sub-cultivation ratio should maintain the cells between 0.2 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 ~2 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.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

- The following assays were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The assay should be performed at least in triplicate.
- Assay A and C should include "Stimulated Cells", "Background Control" (cell-free wells) and "Unstimulated Control" conditions.
- Assay B should include "Background Control", "No Inhibitor Control", "No Inhibitor, No Agonist Control" and "Test Inhibitor" conditions.

Assay Medium: Thaw Medium 2 (BPS Bioscience #60184)

A. Activation of NF-κB Luciferase Reporter Jurkat Cell Line by agonists

1. Seed NF-κB Luciferase Reporter Jurkat cells at ~40,000 cells per well in 50 µl of Thaw Medium 2 into white, clear-bottom 96-well plate. Leave wells without cells for determination of background luminescence ("Background Control").

2. Prepare a three-fold serial dilution of agonist (TNFα or PMA + Ionomycin) at a concentration 2-fold higher than the desired final concentration, in Assay Medium (50 µl/well). If performing an EC_{50} dose response curve to TNFα, we recommend a range of 0.01 to 300 ng/ml. For an EC_{50} dose response curve to PMA, we recommend a range of 0.01 to 100 nM in the presence of 1 µM Ionomycin.

   Note: If the agonist of interest is soluble in DMSO, the final DMSO concentration should not exceed 0.5%.

3. Add 50 µl of diluted agonist to each "Stimulated Cells" well (final volume is 100 µl).

4. Add 50 µl of Assay Medium with the same concentration of DMSO as was used for the dilution of the agonist, to measure the signal in the "Unstimulated Control" wells.

5. Add 100 µl of Assay Medium to the "Background Control" wells (for determining background luminescence).

6. Incubate the cells at 37°C in a CO_2 incubator for ~3-6 hours.

7. Add 100 µl of One-Step™ Luciferase reagent per well.

8. Rock at Room Temperature (RT) for ~15 minutes.

9. Measure luminescence using a luminometer.

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

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

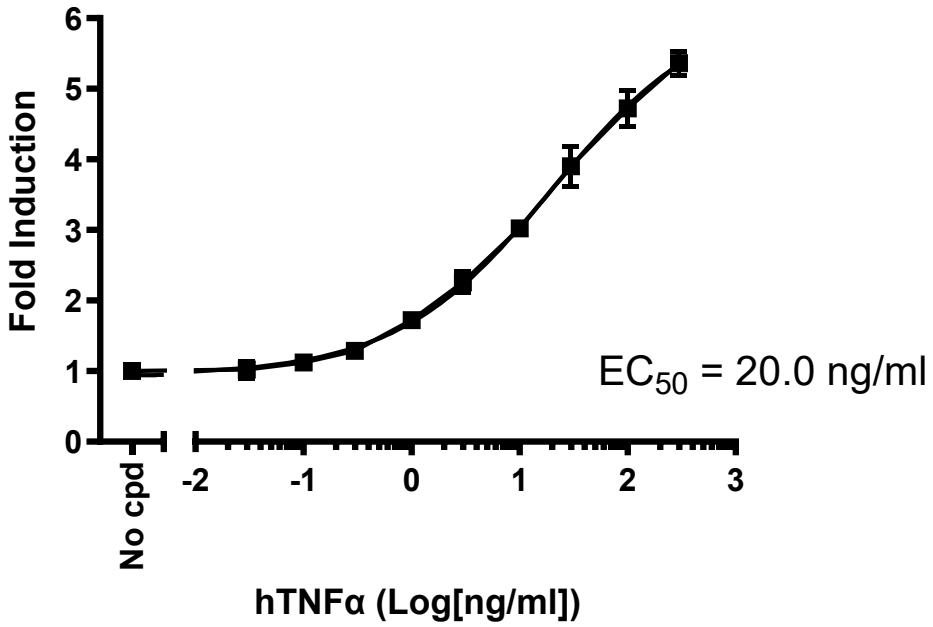

Figure 1: NF-κB Luciferase Reporter Jurkat Cell Line response to TNFα.
Cells were treated with increasing concentrations of TNFα for 6 hours (3-fold serial dilution). The results are shown as fold induction of luciferase reporter expression.

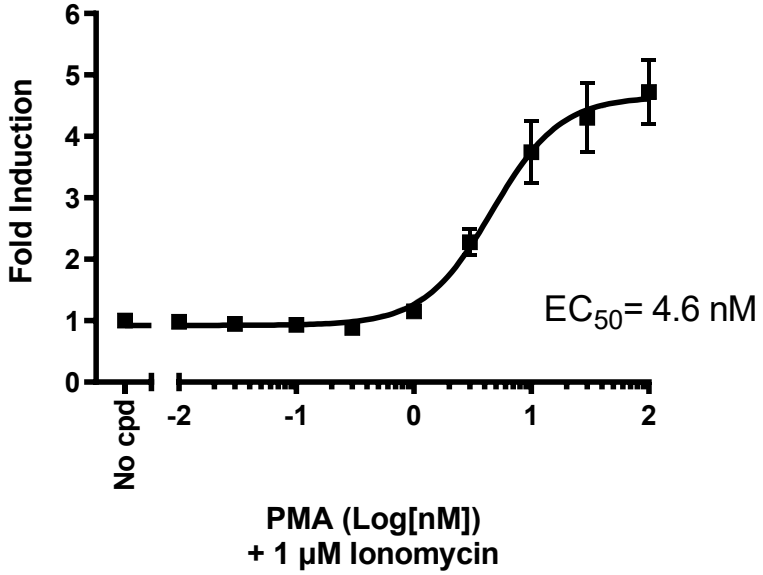

Figure 2: NF-κB Luciferase Reporter Jurkat Cell Line response to PMA + Ionomycin.
Cells were treated with increasing concentrations of PMA plus a fixed concentration of 1 µM ionomycin for 3 hours. The results are shown as fold induction of luciferase reporter expression.

B. Inhibition of agonist-induced NF-kB activity in NF-kB Luciferase Reporter Jurkat cell line

1. Seed NF-kB Luciferase Reporter Jurkat cells at 40,000 cells per well in 50 µl of Thaw Medium 2 into white, clear-bottom 96-well plates. Leave wells without cells for determination of background luminescence ("Background Control").

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

3. Add 25 µl of inhibitor serial dilution to the "Test Inhibitor" wells.

4. Add 25 µl of Assay Medium to the "No Inhibitor Control" and "No Inhibitor, No Agonist Control" wells.

5. Incubate the plate at 37°C with 5% CO_2 for 30 minutes.

6. Prepare a solution of agonist 4-fold higher than the desired final concentration in Assay Medium.

7. Add 25 µl of agonist to the "Test Inhibitor" and "No Inhibitor" wells.

8. Add 25 µl of Assay Medium to the "No Inhibitor, No Agonist" wells.

9. Add 100 µl of Assay Medium to the "Background Control" wells (for determining background luminescence).

10. Incubate the plate 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 RT for ~15 minutes.

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all 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%.

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

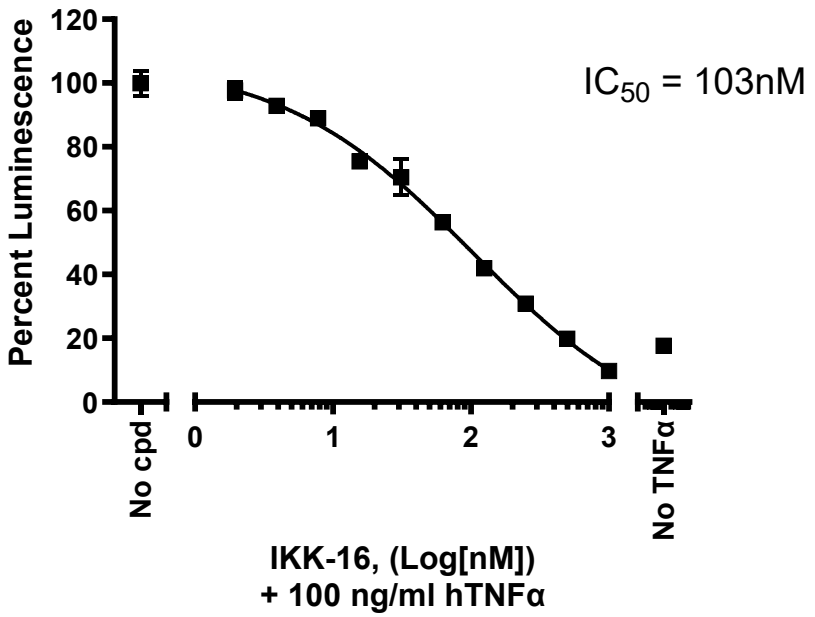

Figure 3: Inhibition of TNFα-induced NF-κB activity by the NF-κB inhibitor IKK-16 dihydrochloride, in NF-κB Reporter Jurkat Cell Line.
NF-κB Reporter Jurkat cells were pre-incubated with increasing doses of IKK-16 prior to stimulation with 100 ng/ml of hTNFα, as described in the protocol above. The results are shown as percent luminescence compared to wells without IKK-16 (set at 100%).

C. Activation of NF-κB Luciferase Reporter Jurkat Cell Line by co-stimulation with anti-CD3 and anti-CD28 antibodies

1. Seed NF-κB Luciferase Reporter Jurkat cells at ~40,000 cells per well in 50 µl of Thaw Medium 2 into white, clear-bottom 96-well plate. Leave wells without cells for determination of background luminescence ("Background Control").

2. Incubate the plate at 37°C in a CO_2 incubator overnight (~18 hours).

3. The next day, prepare a three-fold serial dilution of anti-CD3 antibody at a concentration 4-fold higher than the final desired concentration, in Assay Medium. For an EC_{50} dose response curve, we recommend a range from 0.0003 to 10 µg/ml.

4. Add 25 µl of serially diluted anti-CD3 antibody 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 the "Background Control" wells (for determining background luminescence).

7. Immediately after anti-CD3 addition, add 25 µl of anti-CD28 antibody at 4 µg/ml (final concentration on cells is 1 µg/ml) to the "Stimulated Cells" wells.

8. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

9. Add 100 µl of One-Step™ Luciferase reagent per well.

10. Rock at RT for ~15 minutes.

11. Measure luminescence using a luminometer.

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

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

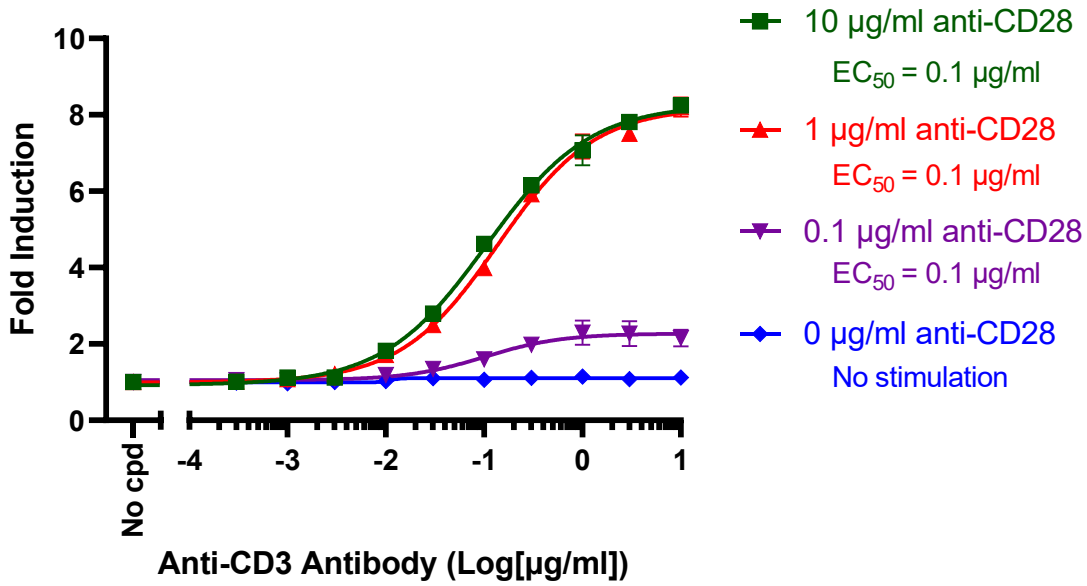

Figure 4: NF-κB Reporter Jurkat Cell Line response to anti-CD3 and anti-CD28 antibodies.
Cells were co-stimulated with increasing concentrations of Anti-CD3 Agonist Antibody in the presence of a fixed concentration of Anti-CD28 Agonist Antibody. Both antibodies are required for stimulation of NF-κB Reporter Jurkat cells.

Data shown is representative.

References

Clipstone N.A. and Crabtree G.R., 1992 *Nature* 357(6380):695-7.
Lyakh, L., *et al.,* 1997 *Mol Cell Biol* 17(5):2475-84.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
NF-κB Luciferase Reporter HEK293 Cell line	60650	2 vials
BCMA/NF-κB Luciferase Reporter HEK293 Cell Line	79755	2 vials
CD27/NF-kB Luciferase Reporter Jurkat Cell Line	79509	2 vials
NF-κB (GFP) Reporter HEK293 Cell Line	79402	2 vials
NF-κB Luciferase Reporter Kit	60614	500 reactions
Firefly Luciferase Jurkat Cell Line	78373	2 vials

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