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Data Sheet

NF-κB Reporter (Luc) - Jurkat Cell line

Catalog #: 60651

Product Description
The NF-κB reporter (Luc)-Jurkat cell line is designed for monitoring nuclear factor Kappa B (NF-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 stimulants of lymphokine receptors, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter gene.

Application
- Monitor NF-κB signaling pathway activity.
- Screen for activators or inhibitors of NF-κB signaling pathway.

Format
Each vial contains ~2 x 10^6 cells in 1 ml of 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of *Mycoplasma* species.

General Culture Conditions
Thaw Medium 2 (BPS Bioscience #60184): RPMI 1640 medium (Thermo Fisher, Cat. #A1049101) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 2B (BPS Bioscience #79530): Thaw Medium 2 plus 1 mg/ml of Geneticin (Thermo Fisher, Cat. #11811031).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2B.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2, **(no Geneticin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2, **(no Geneticin)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 3 – 4 ml of Thaw Medium 2, **(no Geneticin)**. At first passage, switch to Growth Medium 2B **(contains geneticin)**. Cells should be split before they reach 2.5 x 10^6 cells/ml.

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To passage the cells, dilute cell suspension into new culture vessels at no less than $0.1x10^6$ cells/ml. Subcultivation ratio: 1:5 to 1:10 twice a week.

Assay Performance
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.

Materials Required but Not Supplied

- TNFα (Sigma, #T0157-10UG)
- PMA (LC Laboratories, #P1680)
- Ionomycin (Sigma, #I3909)
- Assay Medium: Thaw Medium 2 (BPS Bioscience, #60184)
- Growth Medium 2B (BPS Bioscience, #79530)
- 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- One-Step luciferase assay system (BPS Bioscience, #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

A. TNFα dose response

1. Harvest NF-κB reporter (Luc)-Jurkat cells from culture in Growth Medium 2B and seed cells at a density of ~40,000 cells per well into white clear-bottom 96-well assay plate in 50 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.

2. Prepare threefold serial dilution of TNFα in assay medium. Add 50 µl of diluted TNFα to TNFα-stimulated wells.

3. Add 50 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 ~3-6 hours.

6. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Figure 1. TNFα dose response in NF-κB reporter (Luc)-Jurkat cells. Cells were treated with TNFα for ~ 6 hours. The results were shown as fold induction of luciferase reporter expression.

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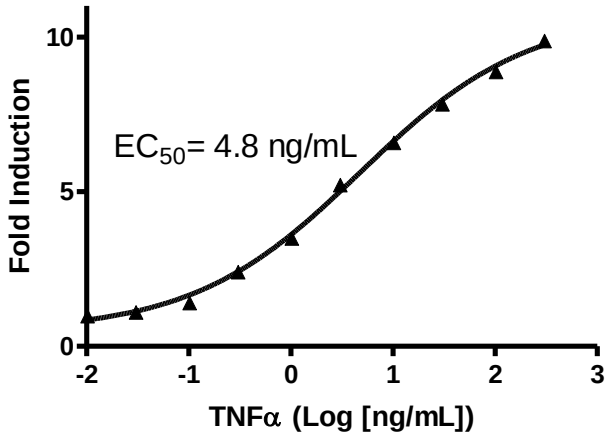
Fold induction was determined by comparing values against the mean value for control cells without TNFα treatment.

The EC50 of TNFα in this cell line is ~4.8 ng/ml.



B. PMA dose response

1. Harvest NF-κB reporter (Luc)-Jurkat cells from culture in Growth Medium 2B and seed cells at a density of ~40,000 cells per well into white clear-bottom 96-well assay plate in 50 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.

2. Dilute ionomycin in assay medium to 4 µM. Prepare threefold serial dilution of PMA in assay medium. Add 25 µl of diluted ionomycin and 25 µl of diluted PMA to stimulated wells (Final concentration of ionomycin 1 µM).

3. Add 50 µl of assay medium with same concentration of DMSO 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 ~3 hours.

6. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Figure 2. PMA dose response in NF-κB reporter (Luc)-Jurkat cells. Cells were treated with PMA plus ionomycin for ~ 3 hours. The results were shown as fold induction of luciferase

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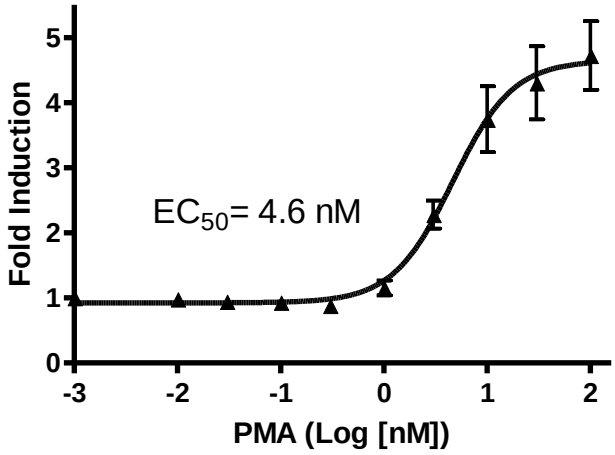
reporter expression. Fold induction was determined by comparing values against the mean value for control cells with DMSO treatment.

The EC50 of PMA in the presence of ionomycin in this cell line is 4.6 nM.



Related Products

Product	Cat. #	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
CD40/NF-κB Reporter (Luc) - HEK293 Cell Line	60626	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Thaw Medium 2	60184	100 ml
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 rxns.

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.

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