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Data Sheet

NF-κB Reporter (Luc) – NIH/3T3 Cell Line

Catalog #: 79469

Product description
The NF-κB reporter (Luc)-NIH/3T3 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, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 5 (BPS Cat. #60182): DMEM (Hyclone #SH30243.01), supplemented with 10% Calf Bovine Serum (Hyclone #SH3007203), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 5A (BPS Cat. #79534): Thaw Medium 5 (BPS Cat. #60186) and 600 µg/ml of Geneticin (Life Technologies #11811031).

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

NF-κB reporter (Luc) – NIH/3T3 cells should exhibit a typical cell division time of 24 hours.

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To thaw the cells, 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 5 **(no Geneticin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 5 **(no Geneticin)**. Transfer the resuspended cells to a T75 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 5 **(no Geneticin)**. At first passage, switch to Growth Medium 5A **(contains Geneticin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 5A and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:5 to 1:10, twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 5A and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

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

Materials Required but Not Supplied

- Mouse TNFα (BioLegend #754402-10 µG)
- Assay medium: Thaw Medium 5 (BPS Bioscience #60182) or
  - DMEM medium (Hyclone #SH30243.01) + 10% Calf Bovine Serum (Hyclone #SH3007203) + 1% Pen/Strep (Hyclone #SV30010.01)
- Growth Medium 5A (BPS Cat. #79534)
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- One-Step luciferase assay system (BPS Bioscience #60690)
- Luminometer

A. TNFα dose response

1. Harvest NF-κB reporter (Luc)-NIH/3T3 cells from culture in Growth Medium 5A and seed cells at a density of ~25,000 cells per well into white opaque 96-well microplate 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.

   Add 50 µl of assay medium to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).

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Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

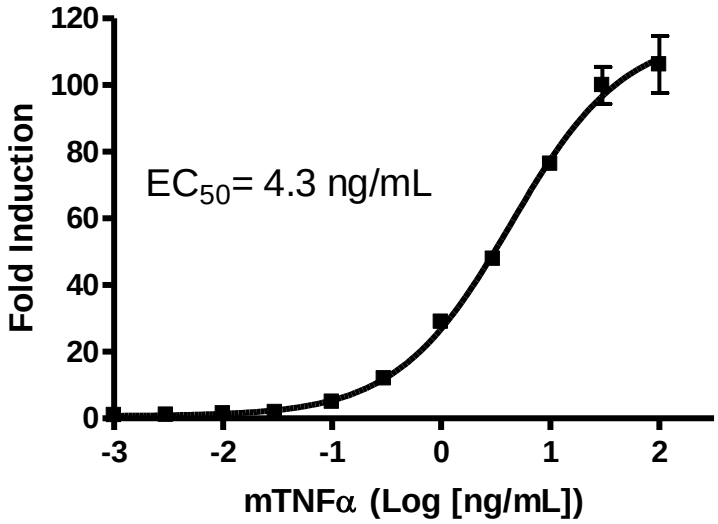
5. Incubate at 37°C with 5% CO_2 for ~3-6 hours.

6. Prepare ONE-Step™ Luciferase Assay reagent as directed. Add 100 µl of ONE-Step™ reagent per well. Incubate at room temperature for ~15 to 30 minutes and 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 = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 1. TNFα dose response in NF-κB reporter (Luc)-NIH/3T3 cells. Cells were treated with TNFα for ~ 6 hours. The results were shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without TNFα treatment.

The EC50 of mTNFα in this cell line is ~4.3 ng/ml.



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Related Products

Product	Cat. #	Size
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB reporter (Luc) - Jurkat Cell line	60651	2 vials
NF-κB reporter (Luc) - CHOK1 Cell line	60622	2 vials
NF-κB reporter (Luc) – A549 Cell line	60625	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
NF-κB Reporter Kit (NF-κb Signaling Pathway)	60614	500 rxns.
Transfection Collection™ :		
NF-κB Transient Pack (NF-κB Signaling Pathway)	79268	500 rxns.
Transfection Collection™ :		
NF-κB Reporter Cellular Assay Pack (HEK293)	79327	2 vials
Transfection Collection™ :		
NF-κB Reporter Cellular Assay Pack (HCT116)	79326	2 vials

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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