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Data Sheet

NF- κB Reporter (Luc) – THP-1 Cell line

Catalog #: 79645

Product Description
The NF-κB reporter (Luc)-THP-1 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 ~5 x 10^6 cells in 1 ml of 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Host Cell
THP-1 Human leukemia monocytic cell line. Non-adherent cells.

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 8 (BPS Bioscience, #79652): RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% heat-inactivated FBS (Life Technologies #10082147), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 8A (BPS Bioscience, #79653): Thaw Medium 8 (BPS Bioscience, #79652) plus 1 µg/ml of Puromycin (Takara, #631306).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 8A.

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 8 **(no Puromycin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 8 **(no Puromycin)**. Transfer the resuspended cells to a T25 flask and incubate at

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37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 1 – 2 ml of Thaw Medium 8 **(no Puromycin)**. At first passage, switch to Growth Medium 8A **(contains Puromycin)**. Cells should be split before they reach 2.0 x 10^6 cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.5 x 10^6 cells/ml. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml.

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)
- LPS (Invivogen, #tlrl-peklps)
- Assay Medium: Thaw Medium 8 (BPS Bioscience, #79652)
- Growth Medium 8A (BPS Bioscience, #79653)
- 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)-THP-1 cells from culture in Growth Medium 8A and seed cells at a density of ~50,000 cells per well into white opaque 96-well microplate in 90 µl of assay medium.

2. Prepare threefold serial dilution of TNFα in assay medium. Add 10 µl of diluted TNFα to TNFα-stimulated wells.

3. Add 10 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. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

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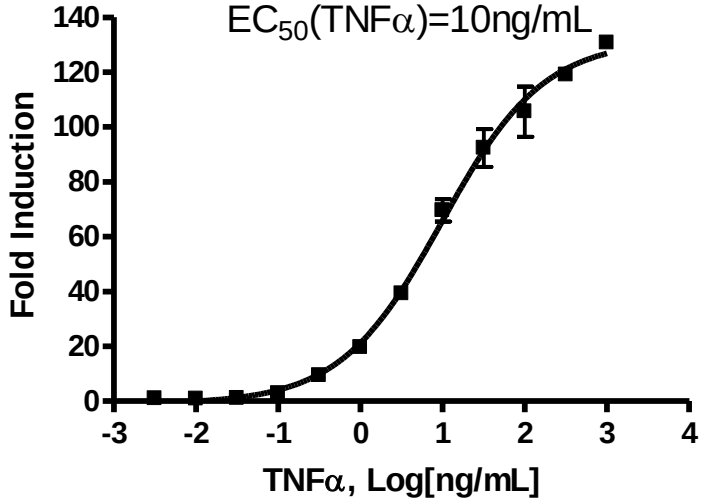
Figure 1. TNFα dose response in NF-κB reporter (Luc)-THP-1 cells. Cells were treated with TNFα for ~ 6 hours. The results were shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without TNFα treatment.

The EC50 of TNFα in this cell line is ~10 ng/ml.



B. LPS dose response

1. Harvest NF-κB reporter (Luc)-THP-1 cells from culture in Growth Medium 8A and seed cells at a density of ~50,000 cells per well into white opaque 96-well microplate in 90 µl of assay medium.

2. Prepare threefold serial dilution of LPS in assay medium. Add 10 µl of diluted LPS to the LPS-stimulated wells.

3. Add 10 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. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15

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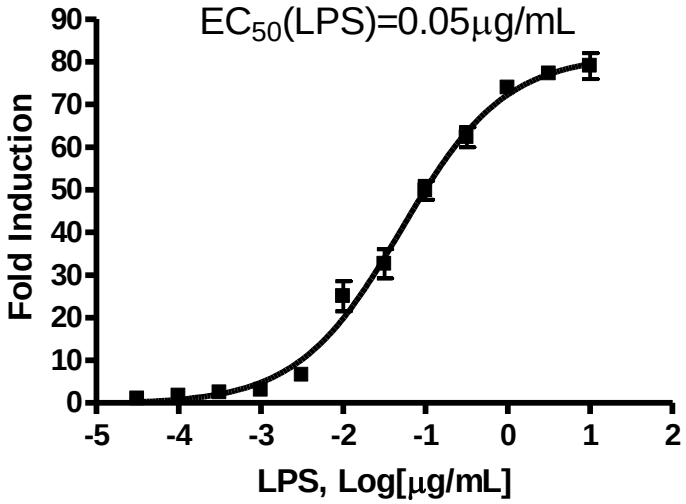
to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

Figure 2. LPS dose response in NF-κB reporter (Luc)-THP-1 cells. Cells were treated with LPS 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.

The EC50 of LPS in this cell line is 0.05 µg/mL.



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
NF-κB Reporter (Luc) - Jurkat Cell Line	60651	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Thaw Medium 8	79652	100 ml
Growth Medium 8A	79653	500 ml
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 rxns.

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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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