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Data Sheet

NF-κB Reporter Kit

NF-κB Signaling Pathway

Catalog #: 60614

Background

NF-κB (Nuclear Factor-KappaB) is a heterodimeric protein composed of different combinations of members of the Rel family of transcription factors. The NF-κB / Rel family of transcription factors (p50, p65, c-Rel, etc.) are involved in stress, immune, and inflammatory responses. In unstimulated cells, the NF-κB dimers are sequestered in the cytoplasm by inhibitory IκB proteins. Proinflammatory cytokines, LPS, growth factors, and antigen receptors activate IκB kinase (IKK), which phosphorylates the IκB proteins. Phosphorylation of IκB leads to its degradation, freeing NF-κB complexes to translocate to the nucleus, bind to NF-κB DNA response elements, and induce the transcription of the target genes.

Description

The NF-κB Reporter kit is designed for monitoring the activity of the NF-κB signaling pathway in the cultured cells. The kit contains transfection-ready NF-κB luciferase reporter vector. This reporter contains a firefly luciferase gene under the control of multimerized NF-κB responsive element located upstream of a minimal promoter. The NF-κB reporter is premixed with constitutively-expressing *Renilla* luciferase vector, whichserves as an internal control for transfection efficiency.

The kit also includes a non-inducible firefly luciferase vector premixed with constitutively-expressing *Renilla* luciferase vector as a negative control. The non-inducible luciferase vector contains a firefly luciferase gene under the control of a minimal promoter, without any additional response elements. The negative control is critical to determining pathway specific effects and background luciferase activity.

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Applications

- Monitor NF-κB pathway activity.
- Screen activators or inhibitors of the NF-κB signaling pathway.
- Study effects of RNAi or gene overexpression on the activity of the NF-κB pathway.

Components

Component	Specification	Amount	Storage
Reporter (Component A)	NF-κB luciferase reporter vector + constitutively expressing *Renilla* luciferase vector	500 µl (60 ng DNA/ µl)	-20°C
Negative Control Reporter (Component B)	Non-inducible luciferase vector + constitutively expressing *Renilla* luciferase vector	500 µl (60 ng DNA/ µl)	-20°C

These vectors are ready-to-use for transient transfection. They are NOT SUITABLE for transformation and amplification in bacteria.

Materials Required but Not Supplied

- Mammalian cell line and appropriate cell culture medium
- 96-well tissue culture plate or 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (Invitrogen # 11668027). However, other transfection reagents work equally well.
- Opti-MEM I Reduced Serum Medium (Invitrogen #31985-062)
- TWO-step luciferase assay system:
  TWO-Step Luciferase (Firefly & Renilla) Assay System (BPS Bioscience #60683): This system assays cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.

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- Luminometer

Generalized Transfection and Assay Protocols

The following procedure is designed to transfect the reporter into HEK293 cells using Lipofectamine 2000 in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine 2000, follow the manufacturer's transfection protocol. Transfection condition should be optimized according to the cell type and study requirements.

All amounts and volumes in the following setup are given on a per well basis.

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection.

2. The next day, for each well, prepare complexes as follows:

a. Dilute DNA mixtures in 15 µl of Opti-MEM I medium (antibiotic-free). Mix gently. Depending upon the experimental design, the DNA mixtures may be any of following combinations:

- **1 µl of Reporter** (component A); in this experiment, the control transfection is **1 µl of Negative Control Reporter** (component B).
- **1 µl of Reporter** (component A) + experimental vector expressing gene of interest; in this experiment, the control transfection is: **1 µl of Reporte**r (component A) + negative control expression vector, **1 µl of Negative Control Reporter** (component B) + experimental vector expressing gene of interest, and **1 µl of Negative Control Reporter** (component B) + negative control expression vector.
- **1 µl of Reporter** (component A) + specific siRNA; in this experiment, the control transfection is: **1 µl of Reporte**r (component A) + negative control siRNA, **1 µl of Negative Control Reporter** (component B) + specific siRNA, and **1 µl of Negative Control Reporter** (component B) + negative control siRNA.

Note: we recommend setting up at least triplicate assays for each condition, and preparing transfection cocktail for multiple wells.

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b. Mix Lipofectamine 2000 gently before use, then dilute 0.35 µl of Lipofectamine 2000 in 15 µl of Opti-MEM I medium (antibiotic-free). Incubate for 5 minutes at room temperature.
Note: Prepare this dilution cocktail in volumes sufficient for the whole experiment.

c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine 2000. Mix gently and incubate for 25 minutes at room temperature.

3. Add the 30 µl of complexes to each well containing cells and medium. Mix gently by tapping the plate.

4. Incubate cells at 37°C in a CO_2 incubator. After ~24 hours of transfection, treat cells with test activators/inhibitors for additional 6 to 24 hours. Perform the TWO-Step Luciferase Assay System following the protocol on the BPS data sheet (BPS Bioscience #60683).

Sample protocol to determine the dose response of HEK293 cells transfected with NF-κB reporter to TNFα

Additional materials required in this experiment setup

- TNFα (Sigma)
- Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01)
- Assay medium: Thaw Medium 1 (BPS Cat. #60187)
- TWO-Step Luciferase (Firefly & Renilla) Assay System (BPS Bioscience #60683)

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into a white clear-bottom 96-well plate in 100 µl of growth medium. Incubate cells at 37°C in a CO_2 incubator for overnight.

2. The next day, transfect 1 µl of NF-κB luciferase reporter (component A) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

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3. After ~24 hours of transfection, dilute TNFα in assay medium and replace cell medium in the stimulated wells with 50 µl of diluted TNFα.
Change medium in the unstimulated control wells to 50µl of assay medium; add 50 µl of assay medium to cell-free control wells (for determining background luminescence).
Set up each treatment in at least triplicate.

4. Incubate cells at 37° in a CO_2 incubator for ~ 6 hours.

5. Perform TWO-step luciferase assay using TWO-Step Luciferase (Firefly & Renilla) Assay System (BPS Bioscience #60683): Dilute 100x Firefly Luciferase Reagent Substrate (Component B) into Firefly Luciferase Reagent Buffer (Component A). Add 50 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C). Add 50 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

7. To obtain the normalized luciferase activity for NF-κB reporter, subtract background luminescence, then calculate the ratio of firefly luminescence from the NF-κB reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

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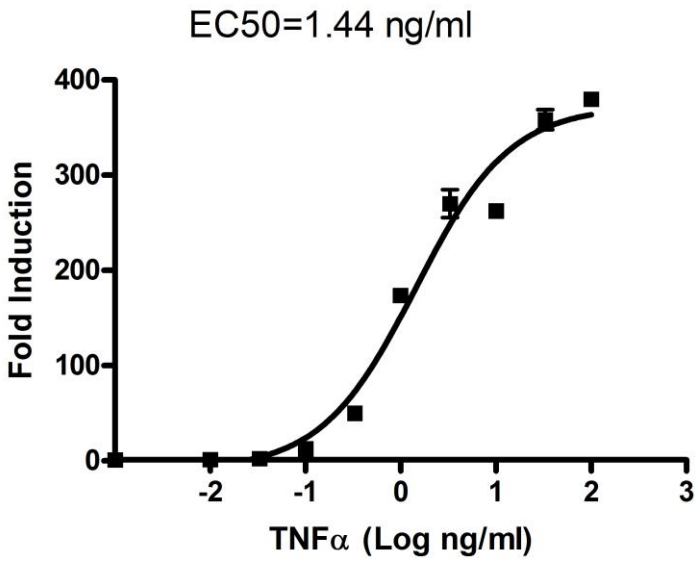
Figure 1. Dose response of NF-κB reporter activity to TNFα in HEK293
The results are shown as fold induction of normalized NF-κB luciferase reporter activity. Fold induction was determined by comparing values against the mean value for control cells without TNFα treatment.

The EC50 of TNFα is ~ 1.44 ng/ml



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-kappa B-like factor to a promoter element. *Mol Cell Biol*. **10(8):**4146-4154.
2. Baeuerle, P.A. (1998). Pro-inflammatory signaling: last pieces in the NF-kappaB puzzle? *Curr Biol*. **8(1):**R19-R22.

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Related products

Product	Cat. #	Size
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-1	10 mL
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-2	100 mL
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-3	1 L
NF-κB Reporter - HEK293 Cell Line	60650	2 vials
TNFα (human)	90244-A	10 µg
TNFα (human)	90244-B	50 µg
TNFα (mouse)	90246-A	5 µg