Rev. 1

ScienCell™
Research Laboratories

NF-kB Dual Reporter Vector

(NF-kB Signaling Pathway)

(NKRV)

Catalog #VT002

100 transfections 96-well

Product Description

The NF-kB signaling pathway (Nuclear Factor kappa-light-chain-enhancer of activated B cells, a family of transcription factors regulating immune and inflammatory responses) coordinates immune responses, inflammation, cell survival, and proliferation, with dysregulation linked to chronic inflammation, autoimmunity, and cancer. NF-kB transcription factors are activated by diverse stimuli (TNF-α, IL-1β, LPS) through upstream kinases (IKK complex), leading to IkB degradation, NF-kB nuclear translocation, and target gene expression. [1,2]. The NF-kB Dual Reporter Vector (NKRV) is a DNA-based dual-luciferase system engineered to monitor this NF-kB–dependent transcriptional activity in mammalian cells. It comprises an NF-kB Reporter Mix, in which Firefly luciferase is driven by multimerized NF-kB response elements and co-formulated with a constitutive Renilla luciferase plasmid for internal normalization, and a Negative Control Reporter Mix lacking NF-kB binding sites to define non-inducible background and pathway specificity. Firefly luciferase provides a strong ATP-dependent signal proportional to NF-kB–driven transcription, while Renilla luciferase offers an ATP-independent internal control, enabling accurate quantification of NF-kB pathway activity suitable for pathway studies, genetic perturbation experiments, and screening of small-molecule or biologic modulators.

Kit Components

Cat #	Component	Quantity	Storage
VT002	NF-kB Reporter Vector Mix (NF-kB Firefly Luciferase Reporter Vector + constitutively Renilla Luciferase Vector), lyophilized	1 vial	-20°C
VTNEG-001	Negative Control Vector Mix (Non-Inducible Firefly Luciferase Vector +constitutively Renilla Luciferase Vector), lyophilized	1 vial	-20°C
GQ100-0.5	Nuclease-free H_2O	1vial (0.5mL)	4°C

Note: These vectors are ready for transient transfection. They are NOT MEANT for transformation and amplification in bacteria.

Additional Materials Required (Materials Not Included in Kit)

Product Name
Mammalian cell line of interest
Appropriate cell culture media

Transfection reagents suitable for your cell type (e.g., UniFectagen Cat#0983)
Nuclease-free H_2O
Cell culture plates (e.g., 6-well, 12-well, 24-well or 96-well plates)
Serum-free or reduced for transfection (e.g., Transfection Max Medium, TMM - Cat#9051)
White or opaque 96-well plate for luminescence reading
Luciferase Assay Kit (LAK, Cat#9048)
Phorbol 12-myristate 13-acetate (PMA)
Recombinant Human Tumor Necrosis Factor-alpha (TNFα, Cat#103-01)
Luminometer

Quality Control

Each batch of NF-kB Dual Reporter Vector (NKRV) undergoes rigorous quality control to optimal performance. Plasmid DNA purity is verified by spectrophotometric, key regulatory elements and luciferase genes are sequence-validated by Sanger sequencing, and functional activity is confirmed via transient transfection in HEK293T cells and ± NF-kB pathway activation (TNFα 10 ng/mL or PMA 20 nM, 6h), followed by Luciferase Assay Kit (LAK, Cat#9048) to verify expected Firefly and Renilla luminescence signals (Figure 1).

Product Use

NKRV is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped at room temperature. Upon receipt, store at -20°C.

References

[1] Oeckinghaus, A., Ghosh, S. The NF-κB Family of Transcription Factors and Its Regulation. *Cold Spring Harb Perspect Biol*. 2009 Oct;1(4): a000034.

[2] Schindler U, Baichwal VR. Three NF-kappa B binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. *Mol Cell Biol*. 1994 Sep;14(9):5820-31.

Procedure

Important Notes:

- *Protocol is optimized for HEK293T cells in a 96-well plate format using UniFectagen transfection reagent (Cat# 0983).*
- *Different reagent: optimize DNA: reagent ratio, incubation time, and complex formation per manufacturer instructions.*
- *Different plate formats: scale volumes and cell numbers by surface area ratios.*
- *Set up ≥3 technical triplicate per condition; prepare transfection cocktails for multiple wells to minimize pipetting errors.*

1. **Cell Seeding**

   Seed approximately 30,000 cells/well in 180 µL complete growth medium in a 96-well plate. Incubate overnight at 37°C in a 5% CO_2.

2. **Plasmid Reconstitution**

2.1. Add 200 µl nuclease-free H_2O (Cat#GQ100-0.5) to **NF-kB Reporter Vector Mix** (lyophilized, Cat#VT002) to make a work solution. Aliquot and store at -20°C (manual defrost freezer). Avoid repeated freeze-and-thaw cycles.

2.2. Add 200 µl nuclease-free H_2O (Cat#GQ100-0.5) to **Negative Control Vector Mix** (lyophilized, Cat#VTNEG-001) to make a work solution. Aliquot and store at -20°C (manual defrost freezer). Avoid repeated freeze-and-thaw cycles.

3. Preparation of DNA Complexes and Cell Transfection

3.1. Prepare Plasmid (per well):

A. NF-kB Reporter (Cat#VT002)
Prepare one well by mixing 2 µL of the reporter plasmid with 5 µL of nuclease-free H_2O or serum free medium (TMM, Cat#9051).

B. Negative Control (Cat#VTNEG-001)
Prepare a separate well by mixing 2 µL of the control plasmid with 5 µL of nuclease-free H_2O or serum free medium (TMM, Cat#9051).

4. Prepare Transfection Reagent Mix (per well)

4.1. Add 0.5 µL of UniFectagen reagent B (Cat#0983) to each plasmid prepared in a 1.5mL sterile microcentrifuge tube. Vortex gently and spin down.

4.2. Add 2 µL of UniFectagen Reagent A (Cat#0983) to bring the total transfection mixture volume to 9.5 µL. Vortex for 5 seconds and spin down.

4.3. Incubate the mixtures at room temperature for 20-30 minutes.
Note: The optimal amount of transfection reagent may vary depending on the reagent and cell type; optimization is recommended.

5. Transfection

5.1. Add the 9.5 µL of transfection mixture to each well containing 100 µL fresh culture medium**.**

5.2. Gently mix into the medium, avoid to disturber cells seeding.

5.3. Incubate the cells for 24 hours at 37°C, 5% CO_2 before downstream analysis.
Note: Depending on the cytotoxicity of the transfection reagent and the sensitivity of the cells, a medium change 4–6 hours post-transfection may be performed.

6. Treatment

6.1. Apply treatments according to experimental groups:

- **Group 1:** Fresh medium only (Untreated)
- **Group 2:** Fresh medium + TNFα (10 ng/mL) or PMA (20 nM)

6.2. Incubate for 6 hours at 37°C.

7. Luciferase Assay

7.1. At the chosen time point post-transfection and/or treatment, measure luciferase activity using the Luciferase Assay Kit (LAK, Cat#9048).

7.2. Follow the instructions provided in the kit manual for cell lysis, reagent preparation, and luminescence detection. Be sure to:

- Use a compatible luminometer.
- Normalize per well: Firefly RLU (Relative Luminescence Units) ÷ Renilla

RLU (same well).
- Use Negative Control Vector to assess background/non-specific effects.
- Calculate fold induction as Treatment ÷ Untreated (each reporter separately).

Dose response of NF-kB Dual Reporter Vector to TNF-α and PMA

Day 1 – Cell Seeding

A. Plate 30,000 HEK293T cells/well in a 96-well plate in 180 µL of complete growth medium.

B. Incubate overnight at 37°C, 5% CO_2.

Day 2 – Transfection

A. Prepare two sets of wells: **NF-kB Reporter Mix (Cat#VT002)** and **Negative Control Mix (Cat#VTNEG-001).**

B. Transfect using optimized 96-well conditions with UniFectagen (Cat# 0983) as described in Procedure section.

C. Incubate the cells for 16-24 hours at 37°C.

Day 3 – Treatment

- **Group 1:** Fresh medium only (Untreated)
- **Group 2:** Fresh medium + TNFα (0.1-50 ng/mL)
- **Group 3:** Fresh medium + PMA (1-100 nM)
- Incubate for 6 hours at 37°C.

Day 4 – Luciferase Assay

- Perform assay using Luciferase Assay Kit (Cat#9048) per kit instructions.
- Normalize Firefly RLU ÷ Renilla RLU (same well).
- Calculate fold induction: Treatment ÷ Untreated.

Notes

- *TNFα: Physiologically activates NF-κB pathway via receptor binding → IkB phosphorylation/degradation → nuclear translocation [1-2].*
- *TNFα treatment: Standard method for rapid, robust NF-κB transcriptional activation in cultured cells [1-2].*
- *Use transfection reagent-compatible medium for optimal efficiency/viability.*

Data Analysis

To obtain normalized luciferase activity, calculate per well: Firefly RLU ÷ Renilla RLU (same well). Fold induction = Treatment ÷ Untreated (for each reporter separately).

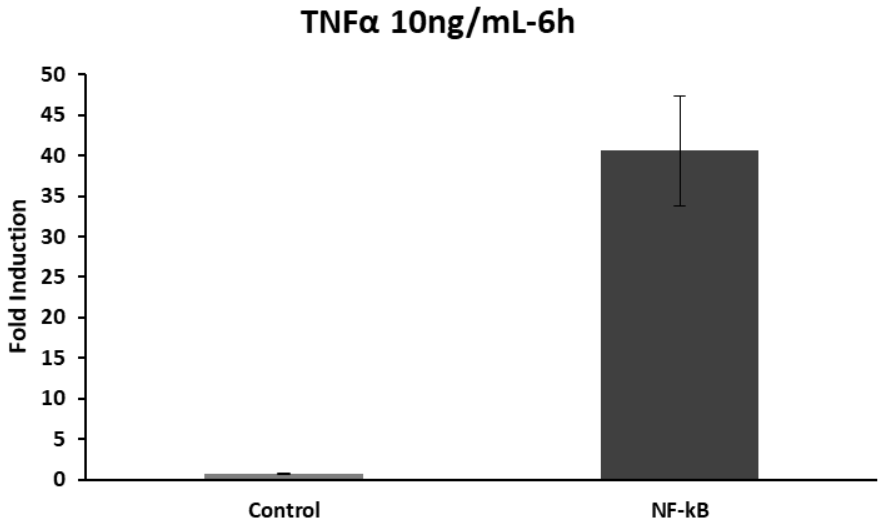

Figure 1. HEK293T cells transiently transfected with NF-kB Dual Reporter Vector, treated 6h with TNF-α (10 ng/mL). Firefly/Renilla normalized luciferase activity (mean ± SD, triplicates).

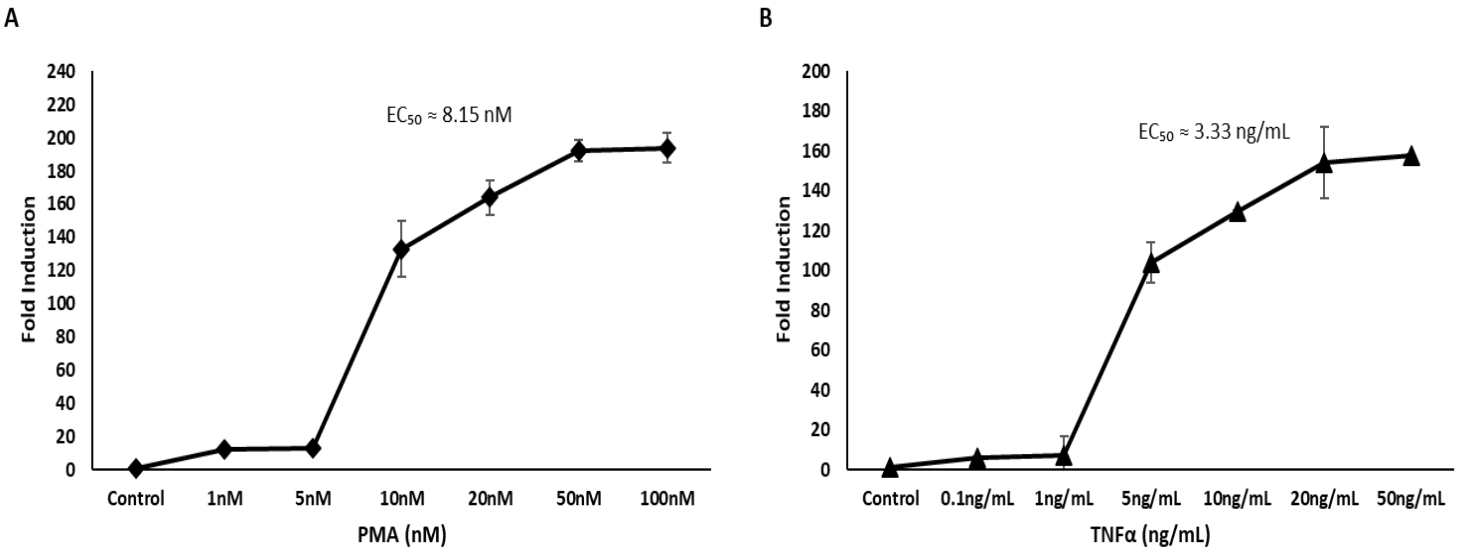

Figure 2. HEK293T cells transfected with NF-kB Dual Reporter Vector, treated 6h with indicated concentrations of PMA (**A**) and TNF-α (**B**). Firefly/Renilla normalized luciferase activity shows dose-dependent NF-kB reporter induction by both agonists (mean ± SD, triplicates).