ScienCell™ Research Laboratories

NF-kB GFP Reporter Vector

(NF-kB Signaling Pathway)

(NK-GFP)

Catalog #VT010

100 transfections 96-well

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) is a central regulator of inflammation, innate immunity, cell survival, and stress responses. In resting cells, NF-kB transcription factors are retained in the cytoplasm by inhibitory IkB proteins (Inhibitor of Nuclear Factor kappa-B). Upon stimulation by cytokines, microbial products, or stress signals, IkB proteins are phosphorylated and degraded, allowing NF-kB to translocate into the nucleus and activate target gene transcription. The NF-kB GFP reporter vector (NK-GFP) is a plasmid-based fluorescent reporter designed for real-time monitoring of NF-kB–dependent transcription in mammalian cells. The vector contains multimerized NF-kB response elements upstream of GFP. Activation of the pathway induces GFP expression, enabling direct visualization of NF-kB activity in living cells without lysis or additional detection reagents [1-3].

Kit Components

Cat #	Component	Quantity	Storage
VT010	NF-kB GFP Reporter 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)
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)
Phorbol 12-myristate 13-acetate (PMA)
Recombinant Human Tumor Necrosis Factor-alpha (TNFα, Cat#103-01)
Fluorescence microscope or fluorescence plate reader

Quality Control

Each batch of NF-kB GFP Reporter Vector (NK-GFP) is verified for plasmid DNA purity and

sequence integrity by spectrophotometry and Sanger sequencing. Functional validation is performed by transient transfection in HEK293T cells followed by NF-kB pathway activation, using TNFα or PMA (TNFα 10 ng/mL or PMA 20 nM, 24h). Inducible GFP expression is confirmed by fluorescence microscopy (Figure 1).

Product Use

NK-GFP 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] Hayden, M. S., & Ghosh, S. (2024). NF-κB in biology and targeted therapy: new insights and perspectives. *Signal Transduction and Targeted Therapy*, 9, 27.

[2] Virchow, C., et al. (2025). NF-κB in inflammation and cancer. *Cellular & Molecular Immunology*, 22, 1310–1328.

[3] Chen, Y., et al. (2017). Development and characterization of a novel NF-κB reporter cell line. *Journal of Neuroinflammation*, 14, 145.

Procedure

Important Notes:

- *Protocol is optimized for HEK293T cells in a 6-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 500,000 cells/well in 2 mL complete growth medium in a 6-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 GFP Reporter Vector** (lyophilized, Cat#VT010) to make a work solution. Aliquot and store at -20°C (manual defrost freezer). Avoid repeated freeze-and-thaw cycles.

3. **Transfection Mix (per well):**

   3.1. Prepare one well by mixing 40 µL of the reporter plasmid with 36 µL of nuclease-free H_2O or serum free medium (TMM, Cat#9051).

   3.2. Add 18 µL UniFectagen Reagent B (Cat#0983). Vortex gently and spin down.

   4.1. Add 48 µL of UniFectagen Reagent A (Cat#0983) to bring the total transfection mixture volume to 142 µL. Vortex for 5 seconds and spin down.

   4.2. Incubate the mixtures at room temperature for 20-30 minutes.

5. **Transfection**
   - **5.1.** Add 142 µL of transfection mixture to each well containing 2 mL 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 24 hours at 37°C.
     Notes: *TNFα: Physiologically activates NF-kB pathway via receptor binding → IkB phosphorylation/degradation → nuclear translocation [1,3].*

7. **Fluorescence Detection**
   - **7.1.** Monitor GFP expression using fluorescence microscopy or a fluorescence plate reader. GFP expression reflects NF-kB pathway activation.

Data Analysis
Increased GFP fluorescence relative to untreated cells indicates activation of NF-kB signaling.

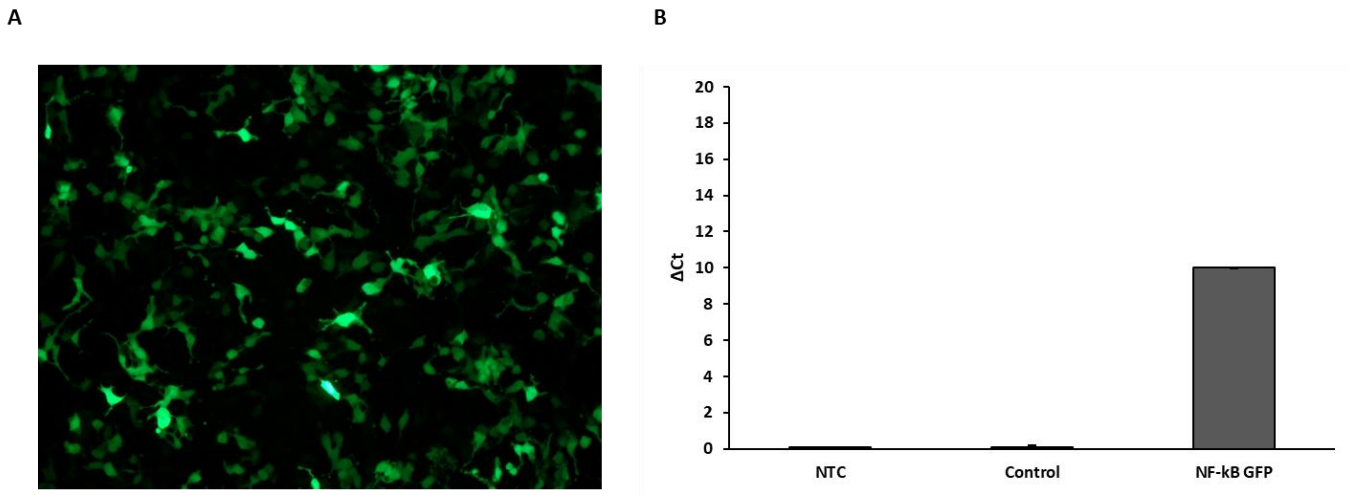


Figure 1. A. Representative fluorescence images of HEK293T cells transfected with the NF-kB GFP Reporter Vector showing NF-kB-dependent GFP expression following pathway activation. **B.** GFP expression was detected by RT-qPCR in transfected HEK293T cells (Ct ~25), with no signal in non-transfected controls. Data were normalized to GAPDH (ΔCt).