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Data Sheet
NF-κB (GFP) – Reporter HEK293 Recombinant Cell Line
Catalog #: 79402

Background

Nuclear factor-κB (NF-κB)/Rel proteins include NF-κB2 p52/p100, NF-κB1 p50/p105, c-Rel, RelA/p65, and RelB. These proteins function as dimeric transcription factors that control genes regulating a broad range of biological processes including innate and adaptive immunity, inflammation, stress responses, B cell development, and lymphoid organogenesis. In the classical (or canonical) pathway, NF-κB/Rel proteins are bound and inhibited by IκB proteins. Proinflammatory cytokines, LPS, growth factors, and antigen receptors activate an IKK complex (IKKβ, IKKα, and NEMO), which phosphorylates IκB proteins. Phosphorylation of IκB leads to its ubiquitination and proteasomal degradation, freeing NF-κB/Rel complexes. Active NF-κB/Rel complexes are further activated by phosphorylation and translocated to the nucleus where they induce target gene expression. In the alternative (or noncanonical) NF-κB pathway, NF-κB2 p100/RelB complexes are inactive in the cytoplasm. Signaling through a subset of receptors, including LTβR, CD40, and BR3, activates the kinase NIK, which in turn activates IKKα complexes that phosphorylate C-terminal residues in NF-κB2 p100. Phosphorylation of NF-κB2 p100 leads to its ubiquitination and proteasomal processing to NF-κB2 p52, creating transcriptionally competent NF-κB p52/RelB complexes that translocate to the nucleus and induce target gene expression.

Description

The NF-κB reporter (GFP)-HEK293 cell line is designed for monitoring the nuclear factor Kappa B (NF-κB) signal transduction pathways. It contains the gene for green fluorescent protein (GFP) driven by four copies of 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 GFP gene.

Applications

The NF-κB reporter cell line is designed for screening activators and inhibitors of NF-κB and for monitoring NF-κB signaling pathway activity.

Format

Two vials containing ~ 1 x 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage

Store in liquid nitrogen immediately upon receipt. Do not store for long-term at -80°C or on dry ice.

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Mycoplasma Testing
This cell line has been screened using the Venor™ GeM Mycoplasma Detection Kit, PCR Based (Sigma, #MP0025) to confirm the absence of Mycoplasma contamination.

Culture Medium:

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, SV30010.01).

Growth Medium 1B (BPS Bioscience, #79531): Thaw Medium 1, 400 µg/ml of Geneticin (Thermo Fisher, #11811031).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1B.

Recommended Culture Condition:

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 **(no Geneticin)**, spin down cells at 1000 rpm, and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 **(no Geneticin)**.Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 1 **(no Geneticin)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 1A **(contains Geneticin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS) and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1B **(contains Geneticin)** and transfer to a tube. Spin down cells, resuspend cells in Growth Medium 1B **(contains Geneticin)** and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:6 to 1:10 weekly or twice a week.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with higher ratio.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (no Geneticin) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots

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into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.

It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

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

Materials Required but Not Supplied

- Thaw Medium 1 (BPS Bioscience, #60187)
- TNFα (BPS Bioscience, # 90244-A)
- 96-well tissue culture plate or 96-well tissue culture-treated black clear-bottom assay plate
- Fluorometer

Assay Protocol

1. Harvest NF-κB (GFP) Reporter – HEK293 cells from culture in growth medium and seed cells into the black clear-bottom 96-well microplate at a density of ~ 35,000 cells per well in 90 µl of Thaw Medium 1. Leave a few of the wells empty for use as a cell-free control.

   Incubate cells at 37°C in a CO_2 incubator overnight.

2. The next day, dilute TNF-α to 1 µg/ml in Thaw Medium 1. Add 10 µl to TNFα-stimulated wells. Add 10 µl of assay medium to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity). Add 100 µl of assay medium to cell-free control wells (for determining background fluorescence).

   Incubate the plate at 37°C in a CO_2 incubator for 24 hours.

3. Read the fluorescence signal at 488 nm excitation and 530 nm emission, or observe under a fluorescence microscope.

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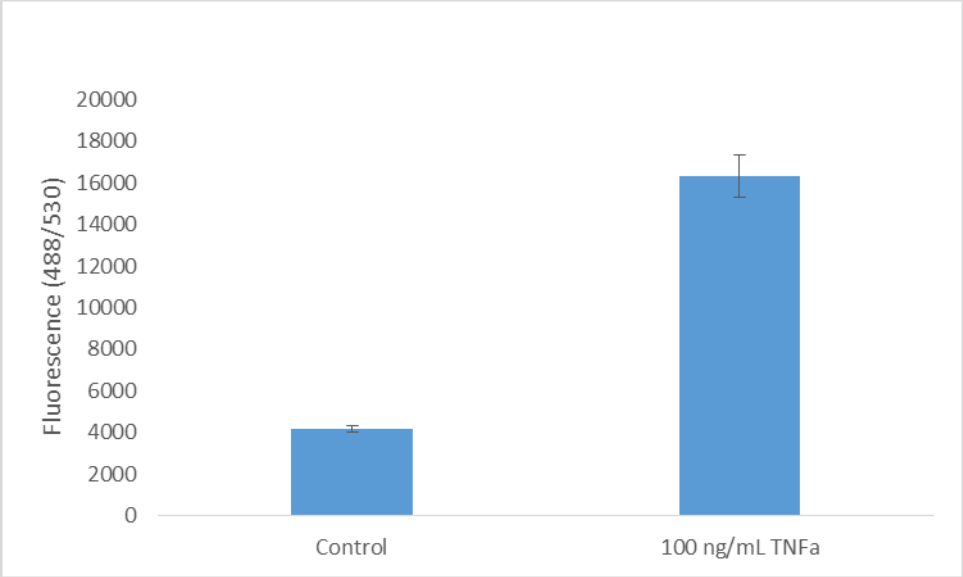

Figure 1. Induction of NF-κB reporter GFP signal by TNFα in HEK293 reporter.

References

1. Pessara U, Koch N (1990). *Mol. Cell Biol.***10**(8):4146-4154.

2. Baeuerle PA (1998). *Curr. Biol.* **8**(1):R19-R22.

3. Takada Y, Kobayashi Y, Aggarwal BB (2005). *J. Biol. Chem.* **280**(17):17203-17212.

Sequence: Jellyfish GFP

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWP
TLVTTFGYGVQCFARYPDHMRQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEG
DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV
QLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMD
ELYK

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Related Products

Product Name	Catalog #	Size
Thaw Medium 1	60187-1	100 mL
Thaw Medium 1	60187-2	500 mL
Growth Medium 1B	79531	500 mL
Human TNF-α	90244-A	10 µL
GFP, His-tag	50277	10 µg
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB Luciferase Reporter (Luc) - Jurkat Cell Line	60651	2 vials