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Data Sheet

NF-kB Reporter (Luc) - A549 Stable Cell Line
Catalog #:60625

Description
NF-κB luciferase reporter construct is stably integrated into the genome of A549 cells. The firefly luciferase gene is controlled by 4 copies of NF-κB response element located upstream of the TATA promoter. Following activation by stimulants, endogenous NF-κB transcription factors bind to the DNA response elements to induce transcription of the luciferase gene.

Background
NF-κB signaling plays a pivotal role in regulating cell development and immune homeostasis. Activation of NF-κB through tumor necrosis factor receptors (TNFR) or the TNFR superfamily member CD40 occurs upon engagement with their respective ligands TNFα or CD40L. Activation of NF-_B enhances cell inflammation and prevents apoptosis, which contribute to tumor development. The A549 lung epithelial cell line is ideal in an *in vitro* lung disease model for high throughput screening of oncogene inhibitors upstream of the NF-κB signaling pathway.

Host Cell
Human alveolar vassal carcinoma cell line. Adherent epithelial cells.

Format
Each vial contains ~3 x 10^6 cells in 1mL of 10% DMSO in FBS.

Storage
Store in liquid nitrogen immediately upon receipt.

Application
The NF-κB-luciferase / A549 cell line is suitable for monitoring the activity of NF-κB transcription factor through luminescence readout instead of using electrophoretic mobility shift assay (EMSA). It provides a platform to enable study of a plethora of signaling pathways upstream of NF-κB in the context of cancer immunology and infectious diseases. NF-κB stimulation by cytokines including TNFa and IL-1b have been validated for this cell line.

Culture Medium
Thaw Medium 6 (BPS #60183): DMEM medium (Hyclone #SH30243.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 6B (BPS #79657): Thaw Medium 6 (BPS #60183) plus 1 mg/mL Geneticin®, G418 Sulfate (Thermo Fisher, Cat. No. 11811031).

Culture Conditions
Frozen Cells: Prepare T-75 culture flask with 20 ml of pre-warmed Thaw Medium 6. Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire content to Thaw Medium 6 (no geneticin).

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Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. 24 hours after incubation, change culture to fresh Thaw Medium 6 (no geneticin); avoid disturbing the attached cells. Continue to monitor growth for 2-3 days and change the media to remove dead cell debris, if necessary. Begin adding Growth Medium 6B after multiple cell colonies (in clumps) start to appear (indicative of healthy cell
division).

Subculture: When cells reached 90% confluency, remove Growth Medium 6B and gently wash cells twice with PBS (without Magnesium or Calcium). Treat cells with 2 ml of 0.25% tryspin/EDTA and incubate for 2-3 minutes at 37°C. Dispense 10 ml of pre-warmed Growth Medium 6B to trypsinized cells and gently pipette up and down to dissociate cell clumps. Transfer cells to a conical tube and centrifuge at 200 x g for 5 minutes. Remove Growth Medium 6B and re-suspend cells in 10 ml of prewarmed Growth Medium 6B. Dispense 2 ml of cell suspension into a new T-75 flask containing prewarmed 18 ml of Growth Medium 6B Incubate cells in a humidified 37°C incubator with 5% CO2. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 90% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Cat. #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Cat. # LT07-518) was used as a positive control.

Quality Assurance

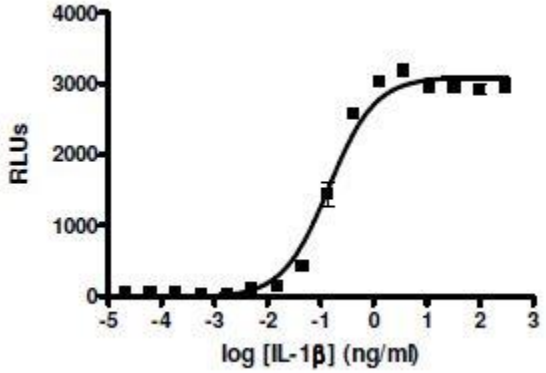

Figure 1. NF-κB/A549 reporter activity in response to IL-1α.
Cells were seeded at 104 cells per well on a white opaque 96- well plate overnight in Growth Medium 6B. Cells were treated with human IL-1α (BPS, Cat. #90168-A) in Growth Medium 6B and incubated for 7 hours at 37°C before the addition of luciferin using the ONE-Step™ Luciferase assay system (BPS, Cat. #60690). Luminescence was read using a luminometer and readings were normalized to wells that only contain media to obtain the Relative Luminescence Unit (RLU). Error bar = standard deviation (SD), n=3. EC50 = 0.142 ng/mL

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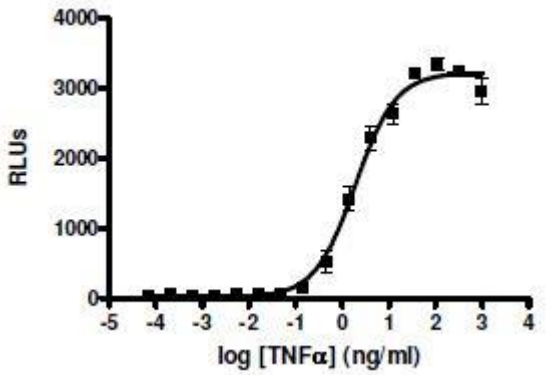

Figure 2. NF-κB/A549 reporter activity in response to TNFα.
Cells were seeded at 104 cells per well on a white opaque 96-well plate overnight in complete growth media (DMEM with 10% FBS and G418). Cells were treated with human TNFα (BPS, Cat. #90244) in growth medium and incubated for 7 hours at 37°C followed by the addition of luciferin using the ONE-Step™ Luciferase assay system (BPS, Cat. #60690). Error bar = standard deviation (SD), n=3. EC50 = 0.282 ng/mL

Vector
NF-κB-Luciferase was cloned into pCDNA3.1™ (+) vector (Invitrogen, Cat. No. V79020).

Application References

1. Cherfilis-Vicini J. *et.al.* (2010) Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. *J. Clin. Invest.* **120:** 1285-1297.
2. Chen W. *et.al.* (2011) NF-_B, a mediator for lung carcinogenesis and a target for lung cancer prevention and therapy. *Front. Biosci.* **16:** 1172-1185.
3. Callister ME *et.al.* (2008) PMX464, a thiol-reactive quinol and putative thioredoxin inhibitor, inhibits NF-_B-dependent proinflammatory activation of alveolar epithelial cells. *Br. J. Pharm.* **155:** 661-672.
4. Schmeck B *et.al.* (2007) Legionella pneumophilia-induced NF-_B- and MAPK-dependent cytokine release by lung epithelial cells. *Eur. Respir. J.* **29:** 25-33

Related Products_

Product	**Cat. #**	**Size**
ONE-StepTM Luciferase Assay System	60690-1	10 ml
ONE-StepTM Luciferase Assay System	60690-2	100 ml
NF-_B reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-_B reporter (Luc) - HCT116 Cell line	60623	2 vials
NF-_B reporter (Luc) - CHO-K1 Cell line	60622	2 vials
NF-_B reporter kit 60614 500 rxns.	60614	500 rxns