6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

NF-κB-Luciferase Reporter HCT-116 Cell Line
Catalog #60623

Description
NF-κB luciferase reporter construct is stably integrated into the genome of HCT-116 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.

Application
The NF-κB-luciferase/HCT-116 cell line is suitable for monitoring the activity of NF-κB signaling in response to stimulants such as the cytokines TNFα and IL-1β, pathogen-associated molecular pattern (PAMP) (i.e. flagellin) or endogenous damage-associated molecular pattern (DAMP) molecules (i.e. NOD1 ligand) (see application references). It is also suitable for establishing cell-based screens for inhibitors that target specific NF-κB stimulating molecules. This cell line can be further modified to allow investigation of downstream NF-κB activities as a result of targeted genetic mutation(s).

Host Cell
HCT-116 Human Colorectal Carcinoma Cell line. Adherent epithelial cells.

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

Storage
Store in liquid nitrogen immediately upon receipt.

Culture Medium

Thaw Medium 7 (BPS #60185): McCoy's 5A medium (Hyclone #SH30200.01) with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01)

Growth Medium 7A (BPS #79543): Thaw Medium 7 (BPS Cat. #60185) plus 1 mg/ml Geneticin (G418) (Thermo Fisher, Cat. #11811031).

Recommended Culture conditions
Frozen Cells: Prepare T-75 culture flask with 20 ml of pre-warmed Thaw Medium 7 **(no G418)**. Quickly thaw cells in a 37°C water bath with constant and slow agitation. After cleaning the outside of the vial with 70% ethanol, immediately transfer the entire content to Thaw Medium 7 **(no G418)**. 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. 24-48 hours after incubation,

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change to fresh Growth Medium 7A **(contains G418)**, without disturbing the attached cells. Continue to change medium every 2-3 days until cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture.

Subculture: When cells reached 90% confluency, remove the medium and wash twice with PBS (without Magnesium or Calcium). Treat cells with 2-3 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 mL pre-warmed medium and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and resuspend cells in 10 ml pre-warmed growth medium. Dispense 2 mL of the cell suspension into a new T75 flask containing pre-warmed 18 ml complete medium (a subcultivation ratio of 1:2 to 1:10 is recommended). Incubate cells in a humidified 37°C incubator with 5% CO_2. To freeze cells, re-suspend cell pellet in freezing medium (10% DMSO in FBS). 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 (Lonza, Cat. #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, Cat. #LT07-518) was used as a positive control.

Application References
1. Samuel T *et.al.* (2014) Variable NF-κB pathway responses in colon cancer cells treated with chemotherapeutic drugs. *BMC Cancer* **14:** 599.
2. Arabi A *et.al*. (2012) Proteomic screen reveals Fbw7 as a modulator of the NF-κB pathway. *Nature Communication* **3:** 976.
3. Clemo NK *et.al*. (2008) BAG-1 is up-regulated in colorectal tumour progression and promotes colorectal tumour cell survival through increased NF-κB activity. *Carcinogenesis* **29:** 849.
4. Tukhvatulin AI *et.al*. (2011) An *In Vitro* and *In Vivo* Study of the ability of NOD1 Ligands to Activate the Transcriptional Factor NF-κB. *Acta Naturae* **3:** 77.

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Quality Assurance and Functional Analysis

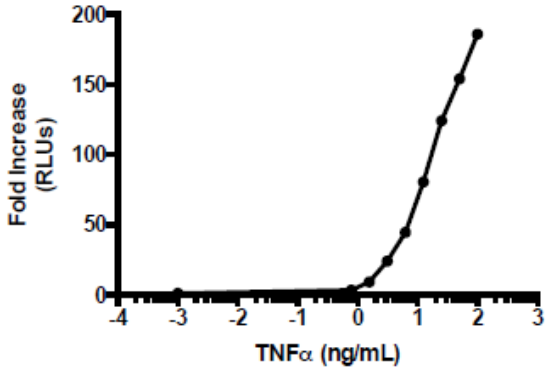

Figure 1. Analysis of NF-κB / HCT-116 reporter activity in response to TNFα. NF-κB-Luciferase HCT-116 cells were seeded on a white opaque 96-well plate overnight at 5000 cells/ well in complete growth medium. Cells were treated with human TNFα in growth medium and incubated for 7 hours at 37°C before the addition of luciferin according to manufacturer's protocol (ONE-Step™ Luciferase assay system, BPS Bioscience, Cat. #60690-2). Luminescence was read using a luminometer and readings were normalized to wells that only contain medium to obtain the Relative Luminescence Units (RLUs). Error bar = standard deviation (SD), n=3.

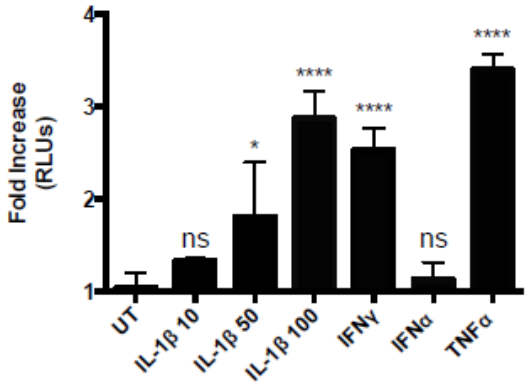

Figure 2. Analysis of NF-κB / HCT-116 reporter activity in response to cytokine stimulation. NF-κB-Luciferase HCT-116 cells were seeded on a white opaque 96-well plate overnight at 5000 cells/ well in complete growth medium. Cells were treated with human cytokines in growth medium (IL-1β, 10, 50, or 100 ng/ml; IFNγ, 2 µg/ml; IFNα, 10^4 U/ml; TNFα, 0.8 ng/ml) and incubated for 7 hours at 37°C, followed by the addition of luciferinaccording to manufacturer's protocol (ONE-Step™ Luciferase assay system, BPS Bioscience, Cat. #60690-2). Luminescence was read using a luminometer and readings were normalized to wells containing only medium to determine the Relative Luminescence Unit (RLU). Fold increase is calculated with respect to untreated control cells (UT). Error bar = standard deviation (SD), n=3. * $P < 0.05$, **** $P < 0.0001$, ns = not significant. One way ANOVA.

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Vector
NF-κB-Luciferase was cloned into the MCS of pCDNA3.1™ (+) vector (Invitrogen, Cat. #V79020).

Related Products

Product	Cat. #	Size
Thaw Medium 7	60185	100 ml
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
NF-κB (Luc) Reporter CHO-K1 Cell Line	60622	2 vials
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 rxns

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