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Data Sheet

NF-κB (Luc) Reporter CHO-K1 Cell Line
Catalog #60622

Description
An NF-κB luciferase reporter construct is stably integrated into the genome of CHO-K1 cells. The firefly luciferase gene is controlled by the 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 / CHO-K1 cell line is suitable for monitoring the activity of NF-κB transcription factor through luminescence readout.). This cell line responds to human cytokine IL-1β, responds moderately to human TNFα, and does not respond to human IFNγ (2 µg/ml). Reducing the amount of serum during incubation period may increase the sensitivity to cytokines. Since CHO-K1 cells do not express endogenous human proteins, this cell line provides an excellent platform to enable exogenous expression of a protein of interest to study its downstream effect on NF-κB signaling.

Host Cell
Chinese Hamster Ovary (CHO)-K1. 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 3 (BPS Cat. #60186): Ham's F-12 medium (Hyclone # SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 3D (BPS Cat. #79539): Thaw Medium 3 (BPS Cat. #60186) 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 3. 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 3 **(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, change to fresh Growth Medium 3D (**contains G418)**, without disturbing the attached cells. Continue to change

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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 reach 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 of pre-warmed Growth Medium 3D 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 3D. Dispense 2 mL of the cell suspension into a new T75 flask containing pre-warmed 18 ml Growth Medium 3D (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 15.

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.

Reference

1. Delude, R.L., *et.al.* (1994) CD14-mediated Translocation of Nuclear Factor-kB Induced by Lipopolysaccharide Does Not Require Tyrosine Kinase Activity. *J. Biol. Chem.* **269:** 22253
2. Railo, A., *et.al.* (2008) Wnt-11 signaling leads to down-regulation of the Wnt/beta-catenin, JNK/AP-1 and NF-kappaB pathways and promotes viability in the CHO-K1 cells. *Exp Cell Res.* **314:** 2389-99
3. Murphy, S.H., *et.al.* (2011) Tumor suppressor protein (p)53, is a regulator of NF-κB repression by the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA* **108:** 17117-17122

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Quality Assurance

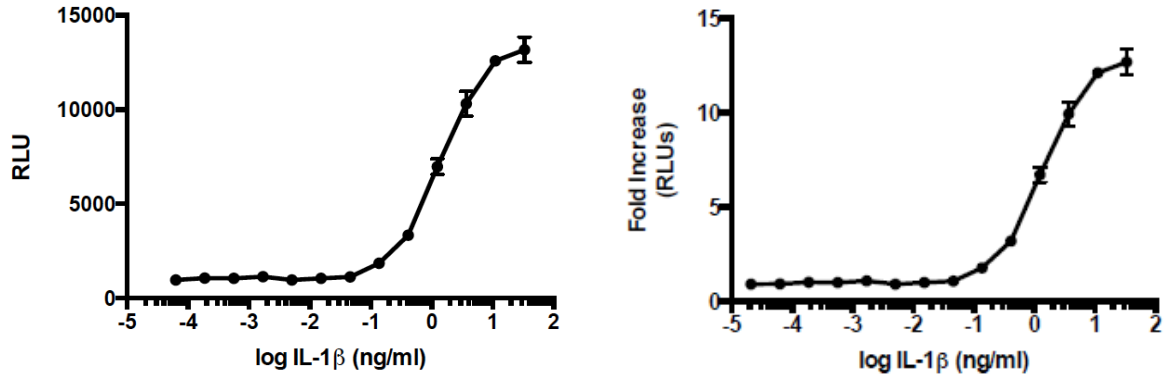

Figure 1. Analysis of NF-κB (Luc) CHO-K1 reporter activity in response to IL-1β.
Cells were seeded at 5000 cells/well on a white opaque 96-well plate overnight in Growth Medium 3D (F-12K with 10% FBS and G418). Cells were treated with human IL-1β in growth medium and incubated for 7 hours at 37°C before 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). Fold Increase was calculated with respect to untreated control cells. Error bar = standard deviation (SD), n=3. EC50 = 10.9 ng/ml

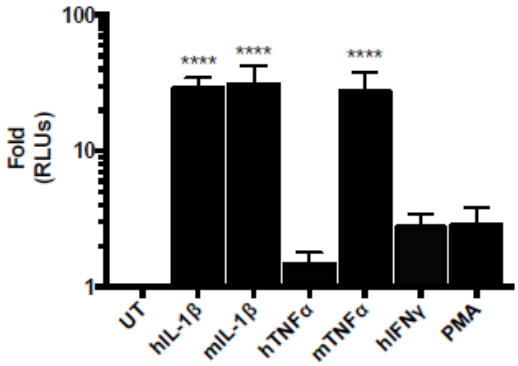

Figure 2. Analysis of NF-κB/CHO-K1 reporter activity in response to various stimuli.
Cells were seeded at 5000 cells/well on a white opaque 96-well plate overnight in serum-free medium. Cells were treated with various human cytokines (IL-17A, 2 µg/ml; IFNγ, 2 µg/ml; TNFα, 20 ng/ml; and PMA, 10 µg/ml) in serum-free medium and incubated for 7 hours, followed by 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

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readings were normalized to wells containing only medium to determine the Relative Luminescence Unit (RLU). Error bar = standard deviation (SD), n=3.

Related Products

Product	Cat. #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
NF-κB Reporter (Luc) - HEK293 Cell Line	60650	2 vials
NF-κB Reporter (Luc) – HCT116 Cell Line	60623	2 vials
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 rxns.

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