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Data Sheet

NF-κB Reporter Cellular Assay Pack (CHO-K1)

Catalog #: 79325

Product Description

The NF-κB Reporter Cellular Assay Pack provides all the key reagents required to monitor the activity of the nuclear factor Kappa B (NF-κB) signal transduction pathways. The pack contains the NF-κB Reporter (Luc)- CHO-K1 Recombinant Cell Line, a luciferase reporter cell line that contains a firefly luciferase gene under the control of four copies of the 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 luciferase reporter gene. This cell line is validated for the response to TNFalpha and to treatment with NF-κB inhibitor, evodiamine.

Additionally, the pack includes cell culture medium (Thaw Medium 3) that has been optimized for use with CHO-K1 cells. Thaw Medium 3 includes 10% fetal bovine serum and 1% Pen/Strep. Finally, the pack provides the ONE-Step™ Luciferase Detection System. This reagent provides highly sensitive, stable detection of firefly (*Photinus pyralis*) luciferase activity. The ONE-Step luciferase reagent can be used directly in cells in Growth Medium 3D and can be detected with any luminometer; automated injectors are not required.

Application

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

Components

Cat. #	Component	Amount	Storage
60622	NF-κB Reporter (Luc) – CHO-K1 Cell Line	2 vials*	liquid nitrogen
60690-1	ONE-Step Luciferase Buffer (**Component A**)	10 ml	-20°C
	ONE-Step Luciferase Reagent Substrate, 100x **(Component B)**	100 µl	-20°C *Protect from light*
60186	Thaw Medium 3	100 ml	+4°C

*Each vial contains ~3 X 10^6 cells in 1 ml of 10% DMSO in FBS.

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General Culture conditions

Thaw Medium 3 (BPS Bioscience #60186): Medium optimized for culturing CHO-K1 cells. Ham's F-12 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 3D (BPS Bioscience #79539): Thaw Medium 3 plus 1 mg/ml Geneticin

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3D (Thaw Medium 3 plus Geneticin). NF-κB reporter (Luc)-CHO cells should exhibit a typical cell division time of 24 hours.

To thaw the cells, prepare a T-75 culture flask with 20 ml of pre-warmed Growth Medium 3D. 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% CO2. 24-48 hours after incubation, change to fresh Growth Medium 3D (**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.
Cells should be split before they reach complete confluence.

To passage the cells, 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 Bioscience recommends preparing frozen stocks so cells are not used beyond passage 15.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 3D and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

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

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Assay performance
The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- IL-1β (BPS Bioscience, #90168)
- Ham's F-12 medium (Hyclone, # SH30526.01)
- TNFα, human (BPS Bioscience, #90244)
- TNFα, mouse (BPS Bioscience, #90246)
- IL-17A (BPS Bioscience, #91014)
- IFN-gamma (BPS Bioscience, #90162)
- Growth Medium 3D (BPS Bioscience, #79539)
- Phorbol 12-myristate 13-acetate (Sigma, #P1585)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- Luminometer

A. IL-1β dose response

1. Harvest NF-κB reporter (Luc)-CHO-K1 cells from culture in Growth Medium 3D and seed cells at a density of 5,000 cells per well into white opaque 96-well microplate in 45 µl of Thaw Medium 3 (no G418).

2. Incubate cells at 37°C with 5% CO_2 overnight.

3. The next day, prepare threefold dilutions of IL-1β in Thaw Medium 3 and add 5 µl to IL-1β-stimulated wells.

   Add 5 µl of Thaw Medium 3 to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).
   Add 50 µl of Thaw Medium 3 to cell-free control wells (for determining background luminescence).

4. Incubate at 37°C with 5% CO_2 for 7-8 hours.

Perform the luciferase detection assay using the ONE-Step™ Luciferase Assay System according to the protocol below:

Luciferase Detection Procedure

5. Thaw Luciferase Reagent Buffer (**Component A**) by placing the reagent in a room temperature water bath. Equilibrate the buffer to room temperature and mix well before use. Note: Luciferase Reagent Buffer must be at ~ room temperature (20- 25°C) before use.
6. Calculate the amount of Luciferase Reagent needed for the experiment (**Component A** + **Component B**). Immediately prior to performing the experiment, prepare the luciferase

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assay working solution by diluting Luciferase Reagent Substrate (**Component B)** into Luciferase Reagent Buffer (**Component A)** at a 1:100 ratio and mix well. Avoid exposing to excessive light. *Only use enough of each component for the experiment, remaining **Component A** and **Component B** should be stored separately at* -20°C.

7. Remove multi-well plate containing mammalian cells from incubator. *Note: plates must be compatible with luminescence measurement with luminometer being used.*
8. Add 50 µl of luciferase assay working solution (**Component A** + **Component B**) to the culture medium in each well.
9. Gently rock the plates for ≥15 minutes at room temperature. Measure firefly luminescence using a luminometer.

The signal under these conditions will be stable for more than 2 hours at room temperature. For maximal light intensity, measure samples within 1 hour of reagent addition.

Data Analysis: Background luminescence is a characteristic of luminometer performance, therefore, background luminescence must be subtracted from all readings for accuracy. Obtain the background-subtracted luminescence by NF-κB subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

The fold induction of luciferase reporter expression = background-subtracted luminescence of IL-1β-stimulated well / average background-subtracted luminescence of unstimulated control wells

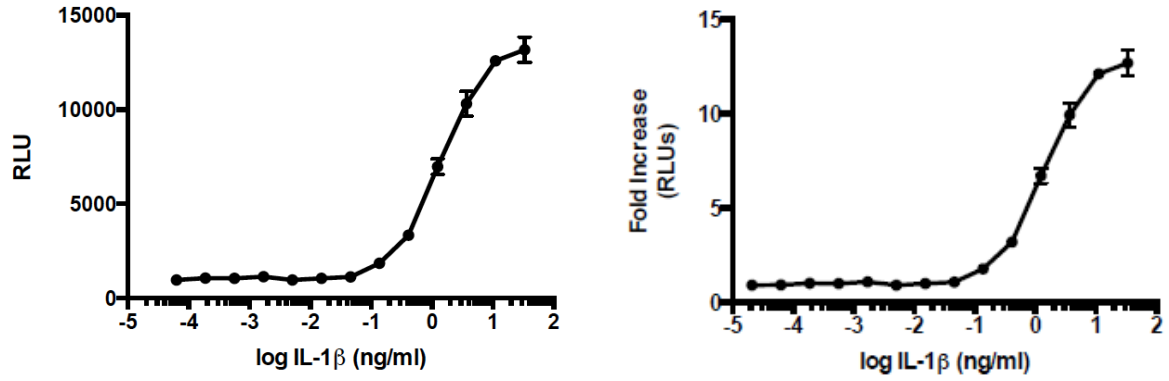

Figure 1. Analysis of NF-κB (Luc) CHO-K1 reporter activity in response to IL-1β.
Cells were seeded at 5000 cells/well on a white opaque 96-well plate overnight in Growth Medium 3D (with G418). Cells were treated with human IL-1β in Growth Medium 3D and incubated for 7 hours at 37°C before the addition of ONE-Step™ Luciferase assay system. 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

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B. Analysis of NF-κB/CHO-K1 reporter activity in response to various stimuli.

1. Harvest NF-κB reporter (Luc)-CHO-K1 cells from culture in Growth Medium 3D and seed cells at a density of 5,000 cells per well into white opaque 96-well microplate in 45 µl of Ham's F-12 medium or other appropriate serum-free medium (assay medium)

2. Incubate cells at 37°C with 5% CO_2 overnight.

3. Add 5 µl of assay medium with cytokine to wells. Incubate cells overnight at 37°C with 5% CO_2. We used IL-17A, 2 µg/ml; IFNγ, 2 µg/ml; TNFα, 20 ng/ml; and PMA, 10 µg/ml.
   Add 5 µl of assay medium to the unstimulated control wells.
   Add 50 µl of assay medium to cell-free control wells.

   Incubate at 37°C with 5% CO_2 for 7-8 hours.

5. Perform the luciferase detection assay using the ONE-Step™ Luciferase Assay System according to the protocol below:

Luciferase Detection Procedure

6. Thaw Luciferase Reagent Buffer (**Component A**) by placing the reagent in a room temperature water bath. Equilibrate the buffer to room temperature and mix well before use. Note: Luciferase Reagent Buffer must be at ~ room temperature (20- 25°C) before use.
7. Calculate the amount of Luciferase Reagent needed for the experiment (**Component A** + **Component B**). Immediately prior to performing the experiment, prepare the luciferase assay working solution by diluting Luciferase Reagent Substrate (**Component B)** into Luciferase Reagent Buffer (**Component A)** at a 1:100 ratio and mix well. Avoid exposing to excessive light. *Only use enough of each component for the experiment, remaining **Component A** and **Component B** should be stored separately at* -20°C.
8. Remove multi-well plate containing mammalian cells from incubator. *Note: plates must be compatible with luminescence measurement with luminometer being used.*
9. Add 50 µl of luciferase assay working solution (**Component A** + **Component B**) to the culture medium in each well.
10. Gently rock the plates for ≥15 minutes at room temperature. Measure firefly luminescence using a luminometer.

The signal under these conditions will be stable for more than 2 hours at room temperature. For maximal light intensity, measure samples within 1 hour of reagent addition.

Data Analysis: Background luminescence is a characteristic of luminometer performance, therefore, background luminescence must be subtracted from all readings for accuracy. Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

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The fold induction of NF-κB luciferase reporter expression = background-subtracted luminescence of cytokine-stimulated well / average background-subtracted luminescence of unstimulated control wells

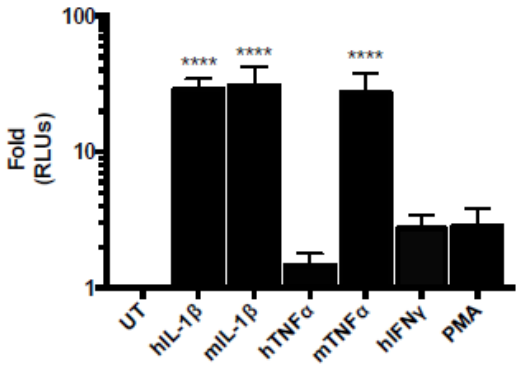

Figure 2. Analysis of NF-κB/CHO-K1 reporter activity in response to various stimuli. Cells were seeded at 5000 cells/well on a white opaque 96-well plate overnight in serum-free medium. Cells were treated with various human cytokines (IL-17A, 2 µg/ml; IFNγ, 2 µg/ml; TNFα, 20 ng/ml; and PMA, 10 µg/ml) in serum-free medium and incubated for 7 hours, followed by the addition of ONE-Step™ Luciferase assay system). Luminescence was read using a luminometer and readings were normalized to wells containing only medium to determine the Relative Luminescence Unit (RLU). Error bar = standard deviation (SD), n=3.

References

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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Refills

Product	Cat. #	Size
NF-κB Reporter (Luc)-CHO-K1 Recombinant Cell Line	60622	2 vials
ONE-Step Luciferase Assay Detection System	60690-1	10 ml
ONE-Step Luciferase Assay Detection System	60690-2	100 ml
ONE-Step Luciferase Assay Detection System	60690-3	1 L
Thaw Medium 3	60186	100 ml

Related Products

Product	Cat. #	Size
NF-κB Reporter (Luc) - Jurkat Recombinant Cell Line	60651	2 vials
NF-κB Reporter (Luc)-HEK293 Recombinant Cell Line	60650	2 vials
NF-κB Reporter (Luc) – A549 Recombinant Cell Line	60625	2 vials
NF-κB Reporter (Luc) - HCT116 Recombinant Cell Line	60623	2 vials
Transfection Collection™ : NF-κB Transient Pack	79268	500 rxns
NF-κB Reporter Kit	60614	500 rxns
TLR9/ NF-κB Reporter – HEK293 Recombinant Cell Line	60485	2 vials
OX40/ NF-κB Reporter – HEK293 Recombinant Cell Line	60482	2 vials
GITR/ NF-κB Reporter – HEK293 Recombinant Cell Line	60546	2 vials
CD40/ NF-κB Reporter – HEK293 Recombinant Cell Line	60626	2 vials
Interleukin-1 beta (IL-1β), human	90168-B	10 µg
TNFα, human	90244-A	10 µg
TNFα, mouse	90246-B	20 µg
IL-17A	91014-2	25 µg
IFN-gamma	90162-A	20 µg