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## Data Sheet

### **Transfection Collection™ - 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**

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

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\*Each vial contains  $\sim 3 \times 10^6$  cells in 1 ml of 10% DMSO in FBS.

### General Culture conditions

**Thaw Medium 3 (BPS Bioscience, #60186):** Medium optimized for culturing CHO-K1 cells. 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 Bioscience, #79539):** Thaw Medium 3 plus 1 mg/ml Geneticin (G418) (Thermo Fisher, Cat. #11811031).

Cells should be grown at 37°C with 5% CO<sub>2</sub> 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% CO<sub>2</sub>. 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<sub>2</sub>. 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.

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### **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..

### **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-1 $\beta$  (BPS Bioscience, #90168)
- Ham's F-12 medium (Hyclone, # SH30526.01)
- TNF $\alpha$ , human (BPS Bioscience, #90244)
- TNF $\alpha$ , 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-1 $\beta$ dose response**

1. Harvest NF- $\kappa$ B 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  $\mu$ l of Thaw Medium 3 (no G418).
2. Incubate cells at 37°C with 5% CO<sub>2</sub> overnight.
3. The next day, prepare threefold dilutions of IL-1 $\beta$  in Thaw Medium 3 and add 5  $\mu$ l to IL-1 $\beta$ -stimulated wells.

Add 5  $\mu$ l of Thaw Medium 3 to the unstimulated control wells (for measuring uninduced level of NF- $\kappa$ B reporter activity).

Add 50  $\mu$ l of Thaw Medium 3 to cell-free control wells (for determining background luminescence).

4. Incubate at 37°C with 5% CO<sub>2</sub> for 7-8 hours.

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

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### 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 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.

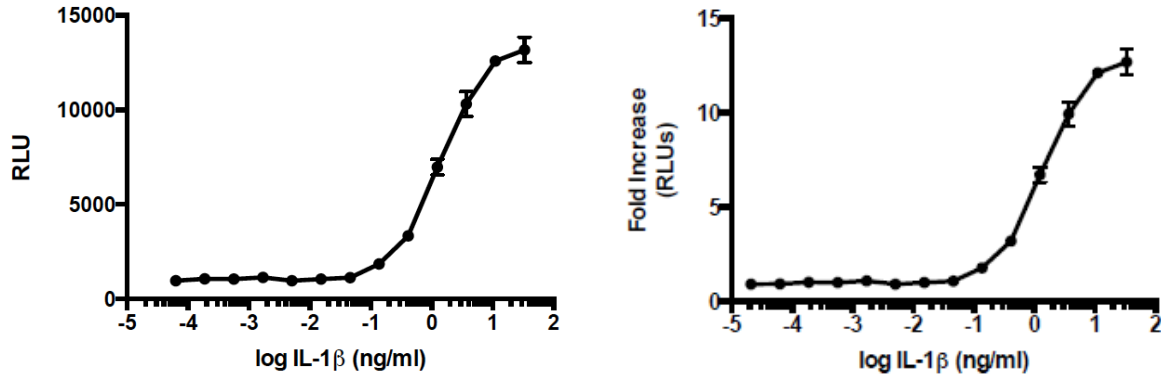
The fold induction of luciferase reporter expression = background-subtracted luminescence of IL-1β-stimulated well / average background-subtracted luminescence of unstimulated control wells

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**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

### 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<sub>2</sub> overnight.
3. Add 5 μl of assay medium with cytokine to wells. Incubate cells overnight at 37°C with 5% CO<sub>2</sub>. 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<sub>2</sub> 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

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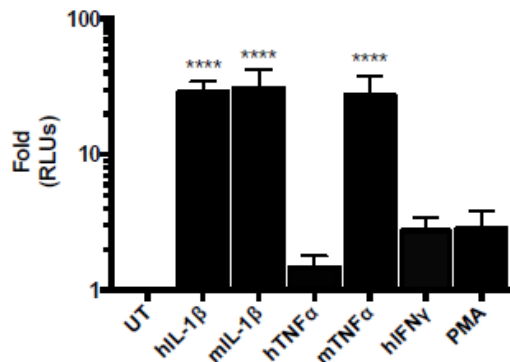
use. Note: Luciferase Reagent Buffer must be at ~ room temperature (20- 25°C) before use.

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.

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,

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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-κB 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-κB 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

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
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

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
NF-κB Reporter (Luc) - Jurkat Recombinant Cell Line	60651	2 vials

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NF- $\kappa$ B Reporter (Luc)-HEK293 Recombinant Cell Line	60650	2 vials
NF- $\kappa$ B Reporter (Luc) – A549 Recombinant Cell Line	60625	2 vials
NF- $\kappa$ B Reporter (Luc) - HCT116 Recombinant Cell Line	60623	2 vials
Transfection Collection™ : NF- $\kappa$ B Transient Pack	79268	500 rxns
NF- $\kappa$ B Reporter Kit	60614	500 rxns
TLR9/ NF- $\kappa$ B Reporter – HEK293 Recombinant Cell Line	60485	2 vials
OX40/ NF- $\kappa$ B Reporter – HEK293 Recombinant Cell Line	60482	2 vials
GITR/ NF- $\kappa$ B Reporter – HEK293 Recombinant Cell Line	60546	2 vials
CD40/ NF- $\kappa$ B Reporter – HEK293 Recombinant Cell Line	60626	2 vials
Interleukin-1 beta (IL-1 $\beta$ ), human	90168-B	10 $\mu$ g
TNF $\alpha$ , human	90244-A	10 $\mu$ g
TNF $\alpha$ , mouse	90246-B	20 $\mu$ g
IL-17A	91014-2	25 $\mu$ g
IFN-gamma	90162-A	20 $\mu$ g

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