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Data Sheet

NF-κB Reporter Cellular Assay Pack (HEK293)
Catalog #: 79327

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)-HEK293 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 1) that has been optimized for use with HEK293 cells. Thaw Medium 1 includes 10% fetal bovine serum, non-essential amino acids, sodium pyruvate, 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 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.

Components

Cat. #	Component	Amount	Storage
60650	NF-κB Reporter (Luc) - HEK293 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*
60187	Thaw Medium 1	100 ml	+4°C

*Each vial contains ~2 X 10^6 cells in 1 ml of 10% DMSO in FBS.

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General Culture conditions

Thaw Medium 1 (BPS Bioscience #60187): MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin .

Growth Medium 1C (BPS Bioscience #79532): MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 50 µg/ml of Hygromycin B

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1C (Thaw Medium 1 plus Hygromycin). NF-κB reporter (Luc)-HEK293 cells should exhibit a typical cell division time of 24 hours.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 **(no Hygromycin)**, spin down cells, resuspend cells in pre-warmed Thaw Medium 1 **(no Hygromycin)**, transfer resuspended cells to a T25 flask and culture in a 37°C CO_2 incubator. At first passage switch to Growth Medium 1C **(contains Hygromycin)**. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. Add Growth Medium 1C and transfer to a tube, spin down the cells, then resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 1C 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 PCR-based VenorGeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

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

- TNFα (BPS Bioscience, #90245)
- IL-1β (BPS Bioscience, #90168)
- Growth Medium 1C (BPS Bioscience, #79532)

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- Evodiamine (Abcam, #142427): inhibitor of NF-κB activation
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- Luminometer

A. TNFα dose response

1. Harvest NF-κB reporter (Luc)-HEK293 cells and seed cells at a density of 30,000 cells per well into white opaque 96-well microplate in 45 µl of Thaw Medium 1 (without Hygromycin).

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

3. The next day, prepare threefold serial dilution of TNFα in Thaw Medium 1 and add 5 µl to TNFα-stimulated wells.
   Add 5 µl of Thaw Medium 1 to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).
   Add 50 µl of Thaw Medium 1 to cell-free control wells (for determining background luminescence).

4. Incubate at 37°C with 5% CO_2 for 5-6 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.

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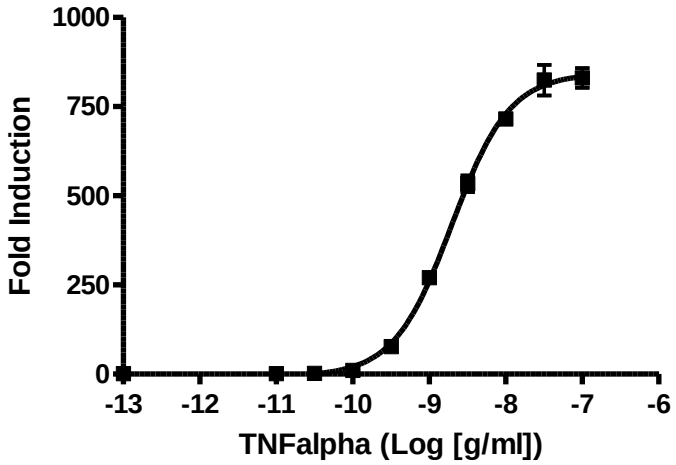
Data Analysis: Background luminescence is a characteristic of luminometer performance, therefore, background luminescence must be subtracted from all readings for accuracy. Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from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

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

Figure 1. TNFα dose response in NF-κB reporter (Luc)-HEK293 cells. The results were shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without TNFα treatment.

The EC50 of TNFα in this cell line is ~2ng/ml.



B. IL-1β dose response

1. Harvest NF-κB reporter (Luc)-HEK293 cells from culture in Growth Medium 1C and seed cells at a density of 30,000 cells per well into white opaque 96-well microplate in 45 µl of Thaw Medium 1 (no hygromycin).

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

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

   Add 5 µl of Thaw Medium 1 to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).

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Add 50 µl of Thaw Medium 1 to cell-free control wells (for determining background luminescence).

5. Incubate at 37°C with 5% CO_2 for 5-6 hours.

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 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 2. IL-1β dose response in NF-κB reporter (Luc)-HEK293 cells. The results were shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without IL-1β treatment.

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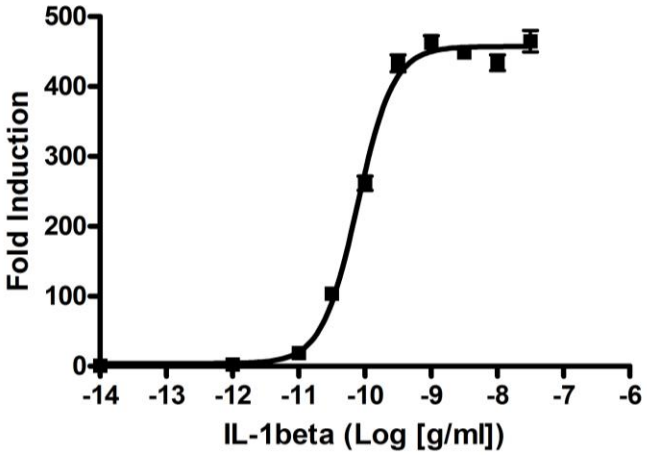
The EC50 of IL-1β in this cell line is ~0.077 ng/ml.



C. Inhibition of TNFα-induced NF-κB activity

1. Harvest NF-κB reporter (Luc)-HEK293 cells from culture in Growth Medium 1C and seed cells at a density of 30,000 cells per well into white opaque 96-well microplate in 45 µl of Thaw Medium 1 (no hygromycin).

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

3. Add 5 µl of Thaw Medium 1 with or without NF-κB inhibitor to wells. Incubate cells overnight at 37°C with 5% CO_2. [Alternately, inhibitor may be added to cells and incubated at 37°C with 5% CO_2 for 2-4 hours before addition of TNFα.]

4. The next day, prepare threefold serial dilution of TNFα in Thaw Medium 1 and add 5 µl to TNFα-stimulated wells.
   Add 5 µl of Thaw Medium 1 to the unstimulated control wells.
   Add 55 µl of Thaw Medium 1 to cell-free control wells.

   Incubate at 37°C with 5% CO_2 for 5-6 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.

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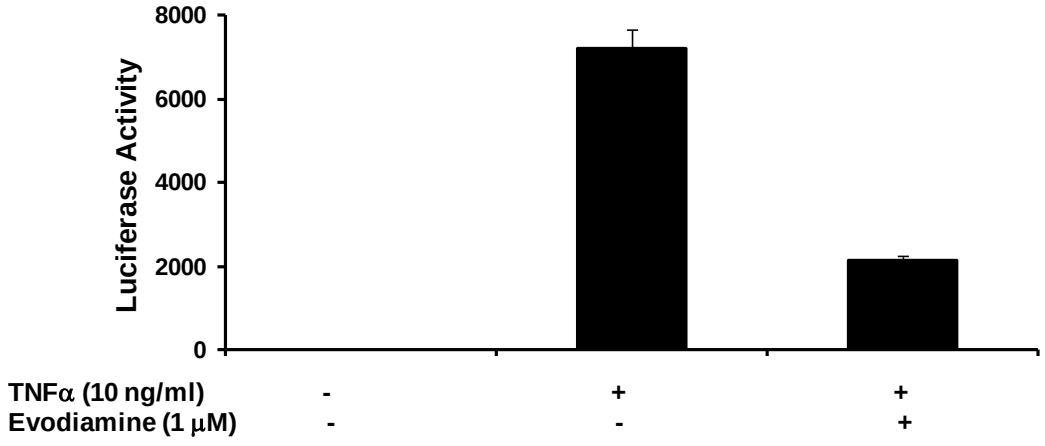
7. Calculate the amount of Luciferase Reagent needed for the experiment (**Component A** + **Component B**). Immediately prior to performing the experiment, prepare the luciferase assay working solution by diluting Luciferase Reagent Substrate (**Component B)** into Luciferase Reagent Buffer (**Component 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 55 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 TNFα-stimulated well / average background-subtracted luminescence of unstimulated control wells

Figure 3. Inhibition of TNFα-induced NF-κB activity by NF-κB inhibitor, evodiamine, in NF-κB reporter (Luc)-HEK293 cells



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References

1. Pessara U, Koch N (1990) Tumor necrosis factor alpha regulates expression of the major histocompatibility complex class II-associated invariant chain by binding of an NF-κB-like factor to a promoter element. *Mol. Cell. Biol*. **10(8):**4146-4154.

2. Baeuerle PA (1998) Pro-inflammatory signaling: last pieces in the NF-κB puzzle? *Curr. Biol*. **8(1):**R19-R22.

3. Takada Y, Kobayashi Y, Aggarwal BB (2005) Evodiamine abolishes constitutive and inducible NF-κB activation by inhibiting IκBα kinase activation, thereby suppressing NF-κB-regulated antiapoptotic and metastatic gene expression, up-regulating apoptosis, and inhibiting invasion. *J. Biol. Chem.* **280(17):**17203-17212

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Refills

Product	Cat. #	Size
NF-κB Reporter (Luc)-HEK293 Recombinant Cell Line	60650	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 1	60187	100 ml

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Related Products

Product	Cat. #	Size
NF-κB Reporter (Luc) - Jurkat Recombinant Cell Line	60651	2 vials
NF-κB Reporter (Luc) - CHO-K1 Recombinant Cell Line	60622	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