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Data Sheet

Transfection Collection™ - NF-κB Reporter Cellular Assay Pack (HCT-116)

Catalog #: 79326

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)- HCT-116 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 7) that has been optimized for use with HCT-116 cells. Thaw Medium 7 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, 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 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).

Components

Cat. #	Component	Amount	Storage
60623	NF-κB Reporter (Luc) – HCT-116 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*
60185	Thaw Medium 7	100 ml	+4°C

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

General Culture conditions

Thaw Medium 7 (BPS Bioscience, #60185): Medium optimized for culturing HCT-116 cells. McCoy's 5A medium (Hyclone, #SH30200.01) with 10% FBS (Life technologies, #26140-079), 1% Penicillin/Streptomycin (Hyclone, SV30010.01).

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

Cells should be grown at 37°C with 5% CO_2 using complete growth medium.

To thaw the cells, prepare a T-75 culture flask with 20 ml of pre-warmed complete growth medium. 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% CO2. 24-48 hours after incubation, change to fresh complete growth medium (**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 complete growth 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 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 complete growth medium 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-1β (BPS Bioscience, #90168)
- TNFα, human (BPS Bioscience, #90244)
- IFN-gamma (BPS Bioscience, #90162)
- IFN-alpha (BPS Bioscience, #90158)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- Luminometer

A. TNFα dose response

1. Harvest NF-κB reporter (Luc)-HCT-116 cells from culture in complete growth medium and seed cells at a density of 5,000 cells per well into white opaque 96-well microplate in 45 µl of Thaw Medium 7 (no G418).

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

3. The next day, prepare threefold dilutions of TNFα in Thaw Medium 7 and add 5 µl to TNFα stimulated wells.

   Add 5 µl of Thaw Medium 7 to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).
   Add 50 µl of Thaw Medium 7 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.

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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 TNFα-stimulated well / average background-subtracted luminescence of unstimulated control wells

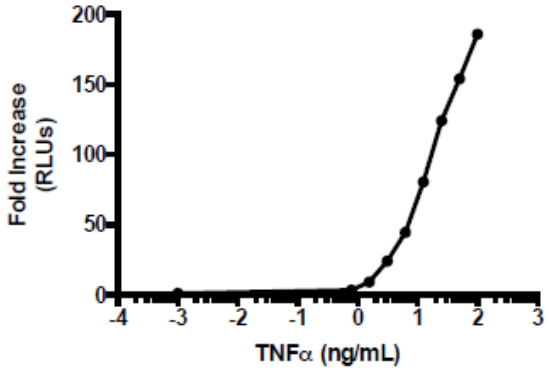

Figure 1. Analysis of NF-κB / HCT-116 reporter activity in response to TNFα. NF-κB-Luciferase HCT-116 cells were seeded on a white opaque 96-well plate overnight at 5000 cells/ well in complete growth medium. Cells were treated with human TNFα in growth medium and

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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). Error bar = standard deviation (SD), n=3.

B. Analysis of NF-κB/HCT-116 reporter activity in response to various stimuli.

1. Harvest NF-κB reporter (Luc)-HCT-116 cells from culture in complete growth medium and seed cells at a density of 5,000 cells per well into white opaque 96-well microplate in 45 µl of Thaw Medium 7 (no G418). Note: Thaw Medium 7 contains 10% fetal bovine serum. Note: For some cytokines, use of serum free medium may result in greater induction of the HCT-116 cells.

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

3. Add 5 µl of Thaw Medium 7 with cytokine to wells. Incubate cells overnight at 37°C with 5% CO_2. We used IL-1β, 10, 50, or 100 ng/ml; IFNγ, 2 µg/ml; IFNα, 10^4 U/ml; TNFα, 0.8 ng/ml.
   Add 5 µl of Thaw Medium 7 to the unstimulated control wells.
   Add 50 µl of Thaw Medium 7 to cell-free control wells.

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

4. 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 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.

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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

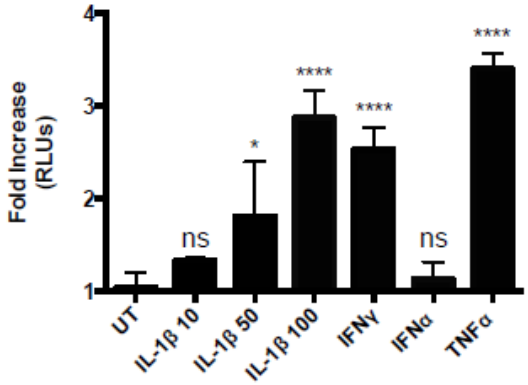

Figure 2. Analysis of NF-κB / HCT-116 reporter activity in response to cytokine stimulation. NF-κB-Luciferase HCT-116 cells were seeded on a white opaque 96-well plate overnight at 5000 cells/ well in complete growth medium. Cells were treated with human cytokines in growth medium (IL-1β, 10, 50, or 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 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). 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.

Application References 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.

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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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Refills

Product	Cat. #	Size
NF-κB Reporter (Luc) - HCT116 Recombinant Cell Line	60623	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 7	60185	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)-CHO-K1 Recombinant Cell Line	60622	2 vials
NF-κB Reporter (Luc) – A549 Recombinant Cell Line	60625	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
IFN-alpha	90158-A	20 µg
IFN-gamma	90162-A	20 µg