Description

The NF-κB reporter (Luc)-HCT-116 cell line is designed to monitor nuclear factor Kappa B (NF-κB) signal transduction pathways. It contains a firefly luciferase gene driven by four copies of the NF-κB response element located upstream of the minimal TATA promoter. After activation by pro-inflammatory cytokines or agonists of lymphokine receptors, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter gene.

Application

- Monitor NF-κB signaling pathway activity 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 references).
- Screen for compound activity of the NF-κB signaling pathway

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~2 x 10^6 cells in 1 ml of 90% FBS, 10% DMSO.

Host Cell

HCT-116 Human Colorectal Carcinoma Cell line. Adherent epithelial cells.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 7	BPS Bioscience #60185
Growth Medium 7A	BPS Bioscience #79543

Materials Required for Cellular Assay

Name	Ordering Information
hTNFα	R&D Systems #210-TA
Assay Medium: Thaw Medium 7	BPS Bioscience #60185
IKK-16 dihydrochloride: inhibitor of NF-κB activation	Sigma #SML1138
96-well tissue culture-treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2. BPS cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

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

Growth Medium 7A (BPS Bioscience #79543):
Thaw Medium 7 (BPS Bioscience #60185) plus 1 mg/ml Geneticin (G418) (Thermo Fisher #11811031).

Assay Medium: Thaw Medium 7 (BPS Bioscience #60185)

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C waterbath, transfer to a tube containing 10 ml of Thaw Medium 7 (**no Geneticin**), spin down cells at 1000 rpm, and resuspend cells in 10 ml of pre-warmed Thaw Medium 7 (**no Geneticin).**
2. Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight.
3. The next day, replace the medium with fresh warm Thaw Medium 7 (**no Geneticin**), and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split.
4. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 7A (**contains Geneticin**).

Cell Passage

1. To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 7A (**contains Geneticin**) and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels.
2. Culture at 37°C in a 5% CO_2 incubator.

Subcultivation ratio: 1:10 to 1:20 weekly.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Validation Data and Assay Performance

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

A. hTNFα dose response

1. Harvest NF-κB reporter (Luc)-HCT-116 cells and seed cells at a density of 5,000 cells per well into a white clear-bottom 96-well microplate in 75 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.
2. Prepare threefold serial dilutions of hTNFα in assay medium at 4x the final concentration and add 25 µl of each dilution to stimulated wells.
3. Add 25 µl of assay medium to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).
4. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
5. Incubate at 37°C with 5% CO_2 for 5-6 hours.
6. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer. If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.
7. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells. The fold induction of NF-κB luciferase reporter expression = background-subtracted luminescence of stimulated wells / average background-subtracted luminescence of unstimulated control wells.

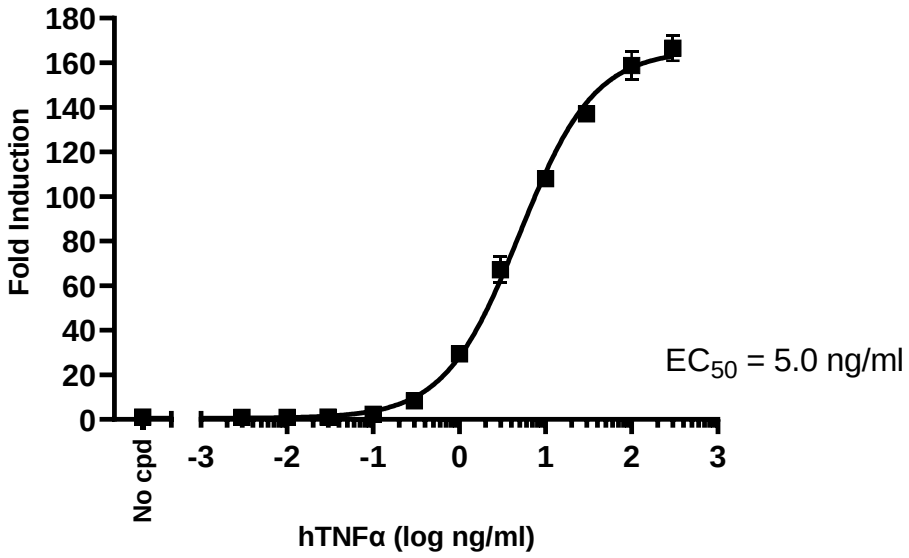

Figure 1. hTNFα dose response in NF-κB reporter (Luc)-HCT-116 cells. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without hTNFα treatment.

B. Inhibition of hTNFα-induced NF-κB activity

1. Harvest NF-κB reporter (Luc)-HCT-116 cells and seed cells at a density of 5,000 cells per well into a white clear-bottom 96-well microplate in 50 µl of assay medium and allow to attach for 4-5 hours.
2. Add 50 µl of assay medium with or without NF-κB inhibitor serial dilutions to wells. We used IKK-16 dihydrochloride (below) as the NF-κB inhibitor. Incubate cells at 37°C with 5% CO_2 overnight. As an alternative, inhibitor can be added to cells the next day and incubated at 37°C with 5% CO_2 for 2-4 hours before addition of hTNFα.
3. Add 30 ng/ml hTNFα in 10 µl of assay medium to hTNFα-stimulated wells.
4. Add 10 µl of assay medium to the unstimulated control wells.
5. Add 110 µl of assay medium to cell-free control wells.
6. Incubate at 37°C with 5% CO_2 for 5-6 hours.
7. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 110 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer. If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol. Subtract background luminescence value from all measurements.
8. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells. The percent luminescence of NF-κB luciferase reporter expression = (background-subtracted luminescence of IKK-16 treated wells / average background-subtracted luminescence of untreated control wells) x 100%.

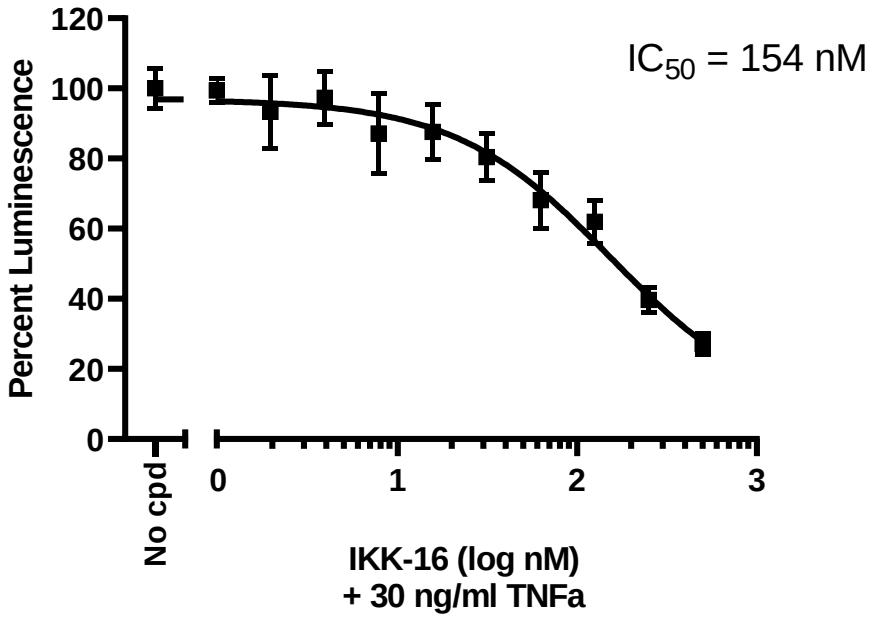

Figure 2. Inhibition of hTNFα-induced NF-κB activity by NF-κB inhibitor, IKK-16 dihydrochloride, in NF-κB reporter (Luc)-HCT-116 cells. The results are shown as percent luminescence compared to wells without added IKK-16.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Thaw Medium 7	60185	100 ml
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
NF-κB (Luc) Reporter CHO-K1 Cell Line	60622	2 vials
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 rxns