SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

NF-κB TWO-Luciferase Reporter HEK293 Cell Line is a HEK293 cell line designed to monitor cell viability alongside NF-κB (nuclear factor Kappa B) activity. It contains a firefly luciferase reporter driven by four copies of the NF-κB response element located upstream of the minimal TATA promoter. In addition, this cell line constitutively expresses Renilla Luciferase under the control of a CMV promoter, which can be used to determine cell viability.

This cell has been validated with TNFα (tumor necrosis factor alpha), and the inhibitor IKK-16 dihydrochloride.

Background

Nuclear factor-Kappa B (NF-κB)/Rel proteins include NF-κB2 p52/p100, NF-κB1 p50/p105, c-Rel, RelA/p65, and RelB. These proteins function as dimeric transcription factors that control genes regulating a broad range of biological processes including innate and adaptive immunity, inflammation, stress responses, B cell development, and lymphoid organogenesis. It is ubiquitously present in almost all mammalian cells, and responds to cellular stress signals, such as stress, free radicals, UV radiation and bacteria and virus. Activation of NF-κB through tumor necrosis factor receptors (TNFR) occurs upon engagement with its respective ligand TNFα (tumor necrosis factor α). Activation of NF-κB enhances cell inflammation and prevents apoptosis, which contribute to tumor development. An understanding of the NF-κB pathway and how to modulate is critical to understand gene regulation in health and disease.

Application

- Monitor NF-kB activity.
- Screen for activators or inhibitors of the NF-kB signaling pathway.
- Study the effect of compound on NF-kB activity and cell viability in parallel.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1G	BPS Bioscience #79544

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
Recombinant Human TNF-alpha Protein	R&D Systems #210-TA/CF
IKK-16 dihydrochloride (inhibitor of NF-κB activation)	Sigma #SML1138
96-well tissue culture-treated white clear-bottom assay plate	Corning #3610
TWO-Step™ Luciferase Assay System	BPS Bioscience #60683
Luminometer	

Storage Conditions

Cells are shipped in 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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

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 1G (BPS Bioscience #79544):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 400 µg/ml of Geneticin and 50 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1G**.**

Cell Passage

1. Aspirate the medium, wash the cells with Phosphate Buffered Saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1G and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1G.

3. Seed into new culture vessels at the desired sub-cultivation ratio of 1:5 to 1:10 weekly or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1G and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

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

Functional Validation

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- Assay A and B should include "Stimulated", "Background Control" and "Unstimulated Control" conditions.
- Assay C should include "Background Control", "No Inhibitor Control", "No Inhibitor, No Agonist Control" and "Test Inhibitor" conditions.

A. NF-κB TWO-Luciferase Reporter HEK293 Cell Line cell titration curve in response to hTNFα.

1. Seed NF-κB TWO-Luciferase Reporter HEK293 cells at varying cell densities in 90 µl of Thaw Medium 1 into a white clear-bottom 96-well cell culture plate. For a cell titration curve, we recommend performing a serial dilution at 2:1 cells to media. Leave a few empty wells as "Background Control" wells.
2. Incubate the cells at 37°C with 5% CO_2 overnight.
3. The next day, prepare a solution of hTNFα in Assay Medium at 10x the final testing concentration (10 µl/well).
4. Add 10 µl of hTNFα to "Stimulated" wells.
5. Add 10 µl of Assay Medium to "Unstimulated Control" wells.
6. Add 100 µl of Assay Medium to "Background Control" wells.
7. Incubate at 37°C with 5% CO_2 for 5-6 hours.
8. Add 100 µl per well of TWO-Step Firefly Luciferase Assay Working Solution.
9. Incubate with gentle agitation at RT for ~15 minutes.
10. Measure Firefly luminescence using a luminometer.
11. On the same plate, add 100 µl per well of TWO-Step Renilla Luciferase Assay Working Solution.
12. Measure Renilla luminescence using a luminometer.
13. Data Analysis: Subtract the respective background luminescence from both the Firefly and Renilla luminescence reading of all conditions.

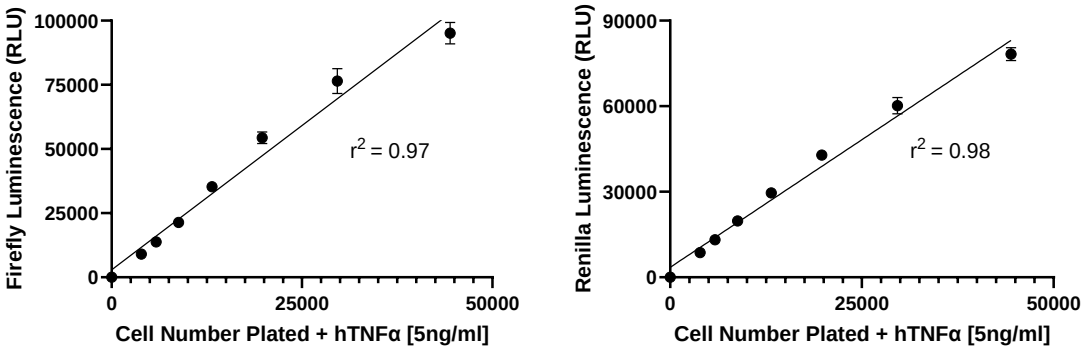

Figure 1. NF-κB TWO-Luciferase Reporter HEK293 Cell Line luciferase activity in a cell titration curve in the presence of hTNFα.
Cells were plated at various densities in a 96-well plate overnight followed by treatment with hTNFα the following morning. Both Firefly and Renilla luciferase activity were measured using TWO-Step Luciferase (Firefly & Renilla) Assay System. Results are shown as background subtracted luminescence signal.

B. Dose Response of NF-κB TWO-Luciferase Reporter HEK293 Cell Line to hTNFα

1. Seed NF-κB TWO-Luciferase Reporter HEK293 cells at a density of 30,000 cells per well into a white, clear-bottom 96-well culture plate in 75 µl of Assay Medium. Leave empty wells as cell-free control wells ("Background Control").
2. Incubate cells at 37°C with 5% CO_2 overnight.
3. Prepare a serial dilution of hTNFα in Assay Medium at 4x the final testing concentrations (25 µl/well).
4. Add 25 µl of each dilution to the wells labeled as "Stimulated".
5. Add 25 µl of Assay Medium to the "Unstimulated Control" wells.
6. Add 100 µl of Assay Medium to "Background Control" wells.
7. Incubate at 37°C with 5% CO_2 for 5-6 hours.
8. Add 100 µl per well of TWO-Step Firefly Luciferase Assay Working Solution.
9. Incubate with gentle agitation at RT for ~15 minutes.
10. Measure Firefly luminescence using a luminometer.
11. On the same plate, add 100 µl per well of TWO-Step Renilla Luciferase Assay Working Solution.
12. Measure Renilla luminescence using a luminometer.

13. Data Analysis: Subtract the respective background luminescence from both the Firefly and Renilla luminescence reading of all conditions. Next, for each well, divide the Firefly luminescence signal by the Renilla luminescence signal.

$$Normalized\ Luminescence = \left(\frac{Firefly\ luminescence\ of\ cells - Firefly\ luminescence\ background}{Renilla\ luminescence\ of\ cells - Renilla\ luminescence\ background}\right)$$

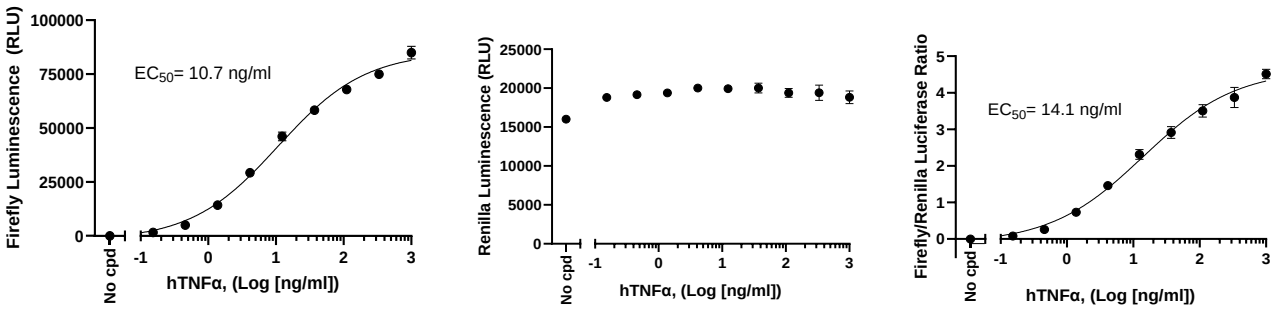

Figure 2. Dose response of NF-κB TWO-Luciferase Reporter HEK293 Cell Line to hTNFα.
The day following cell plating, NF-κB TWO-Luciferase Reporter HEK293 cells were treated with increasing doses of hTNFα for 5 hours. Both Firefly and Renilla luciferase activity were measured using TWO-Step Luciferase (Firefly & Renilla) Assay System. Results are shown as background-subtracted luminescence signal (left and middle graphs) and normalized luminescence signal (right graph).

C. Inhibition of agonist-induced NF-κB activity in NF-κB TWO-Luciferase Reporter HEK293 Cell Line

1. Seed NF-κB TWO-Luciferase Reporter HEK293 cells at a density of 30,000 cells per well into a white, clear-bottom 96-well culture plate in 60 µl of Assay Medium. Leave empty wells as cell-free control wells ("Background Control").

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

3. Prepare a serial dilution of NF-κB inhibitor in Assay Medium at 3x the final testing concentrations (30 µl/well).

4. Add 30 µl of inhibitor to the "Test Inhibitor" wells.

5. Add 30 µl of Assay Medium to the "No Inhibitor Control" and "No Inhibitor, No Agonist Control" wells.

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

7. The next day, prepare solution of agonist in Assay Medium at 10x the final testing concentration (10 µl/well).

8. Add 10 µl of agonist solution to the wells labeled as "Test Inhibitor" and "No Inhibitor Control".

9. Add 10 µl of Assay Medium to the "No Inhibitor, No Agonist Control" wells.

10. Add 100 µl of Assay Medium to the "Background Control" wells.
11. Incubate at 37°C with 5% CO_2 for 5-6 hours.
12. Add 100 µl per well of TWO-Step Firefly Luciferase Assay Working Solution.
13. Incubate with gentle agitation at RT for ~15 minutes.
14. On the same plate, add 100 µl per well of TWO-Step Renilla Luciferase Assay Working Solution.
15. Measure Renilla luminescence using a luminometer.
16. Data Analysis: Subtract the respective background luminescence from both the Firefly and Renilla luminescence reading of all conditions. Next, for each well, divide the Firefly luminescence signal by the Renilla luminescence signal.

$$Normalized\ Luminescence = \left(\frac{Firefly\ luminescence\ of\ cells - Firefly\ luminescence\ background}{Renilla\ luminescence\ of\ cells - Renilla\ luminescence\ background}\right)$$

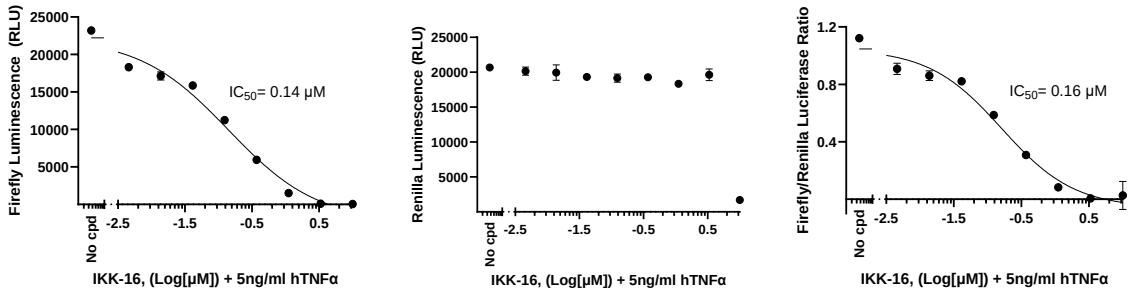

Figure 3. Inhibition of TNFα-induced NF-κB activity by the NF-κB inhibitor IKK-16 dihydrochloride in NF-κB TWO-Luciferase Reporter HEK293 Cell Line.
NF-κB TWO-Luciferase Reporter HEK293 cells were pre-incubated with increasing doses of IKK-16 prior to stimulation with 5 ng/ml of hTNFα, as described in the protocol above. Both Firefly and Renilla luciferase activity were measured using TWO-Step Luciferase (Firefly & Renilla) Assay System. Results are shown as background-subtracted luminescence signal (Left and middle graphs) and normalized luminescence signal (right graph).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Chen W., *et.al*., 2011 *Front. Biosci.* 16: 1172-1185
Schmeck B., *et.al*., 2007 *Eur. Respir. J.* 29: 25-33

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

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*
NF-κB Reporter (Luc) HEK293 Cell Line	60650	2 vials
BCMA / NF-κB – Reporter HEK293 Recombinant Cell Line	79755	2 vials
LIGHT-Responsive HVEM/NF-κB Reporter Jurkat Cell Line	79310	2 vials
CD27/NF-kB Reporter-Jurkat Recombinant Cell Line	79509	2 vials

Version 080124