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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
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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 Luciferase-eGFP Reporter HEK293 Cell Line is a HEK293 cell line designed to monitor nuclear factor Kappa B (NF-κB) activity. It contains a firefly luciferase-eGFP (enhanced green fluorescent protein) reporter driven by multiple copies of the NF-κB response element located upstream of the minimal TATA promoter. After activation by pro-inflammatory cytokines or agonists of the lymphokine receptors, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase-eGFP reporter.

The cell line has been functionally validated in response to human TNF-α.

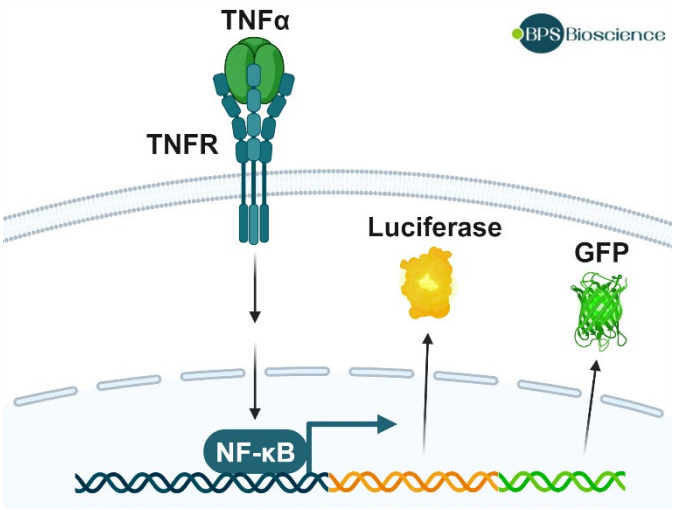

Figure 1: Expression of luciferase and eGFP driven by TNF-α-mediated activation of transcription factor NF-κB in the NF-κB Luciferase-eGFP Reporter HEK293 Cell Line.

Background

The role of NFκB (nuclear factor kappa-light chain enhancer of activated B cells) is well-characterized in canonical (classical) and noncanonical (alternative) signaling pathways of inflammation. Two major forms of innate immune sensors are Toll-like receptors (TLR) and NOD/CATERPILLER proteins. Mutations in NOD2 (nucleotide-binding oligomerization domain-containing protein 2) have been linked to chronic autoinflammatory and autoimmune diseases, such as Crohn's disease and Blaus syndrome. Studying the canonical and noncanonical NF-κB pathways and the influence of TLR pathways and NOD2 mutations can further our understanding of autoimmune regulation. The use of luciferase and eGFP (enhanced green fluorescence protein) reporter allows for easy read outs in cellular assays.

Application

- Monitor NF-κB activity.
- Screen or characterize compound activity on the NF-κB signaling pathway.

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)

Host Cell

HEK293, epithelial-like cells, adherent

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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 1	BPS Bioscience #60187
Growth Medium 1N	BPS Bioscience #79801

Materials Required for Cellular Assays

Name	Ordering Information
Tumor Necrosis Factor-α Human	Sigma #T0157-10UG
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
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 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*. To formulate a comparable but not BPS Bioscience's validated media, formulation components can be found below.

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 1N (BPS Bioscience #79801):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin and 0.5 µg/ml of Puromycin.

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Media Required for Functional Cellular Assay

Assay Medium:

Thaw Medium 1 (BPS Bioscience #60187)

Cell Culture Protocol

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

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath.

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

2. Transfer the contents of the cryovial to a tube containing 10 ml of Thaw Medium 1.

3. 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.

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

5. After 24 hours of culture, check for cell viability and attachment. For a T25 flask, add 3-4 ml of Thaw Medium 1, and continue growing in a 5% CO2 incubator at 37°C until the cells are ready to passage.

6. Cells should be passaged before they reach full confluency. Switch to Growth Medium 1N at first and subsequent passages.

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 1N and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1N.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10-1:20 weekly.

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 1N 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.

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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 down at least 10 vials of cells at an early passage for future use.

Validation Data

- 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 should include "Stimulated", "Background Control" and "Unstimulated Control" conditions.

1. Seed NF-κB Luciferase-eGFP HEK293 cells at a density of 20,000 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 threefold serial dilution of agonist in Assay Medium at concentrations 4-fold higher than the final desired concentrations (25 µl/well).

4. Add 25 µl of each agonist dilution to the wells labeled as "Stimulated".

5. Add 25 µl of Assay Medium to the "Unstimulated Control" wells (for measuring uninduced level of NF-κB reporter activity).

6. Add 100 µl of Assay Medium to "Background Control" wells.

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

8. The expression of luciferase can be measured using the ONE-Step™ Luciferase Assay System (#60690), and the expression of eGFP can be analyzed by flow cytometry or by microscopy.

9. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB luciferase-eGFP reporter expression is the background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

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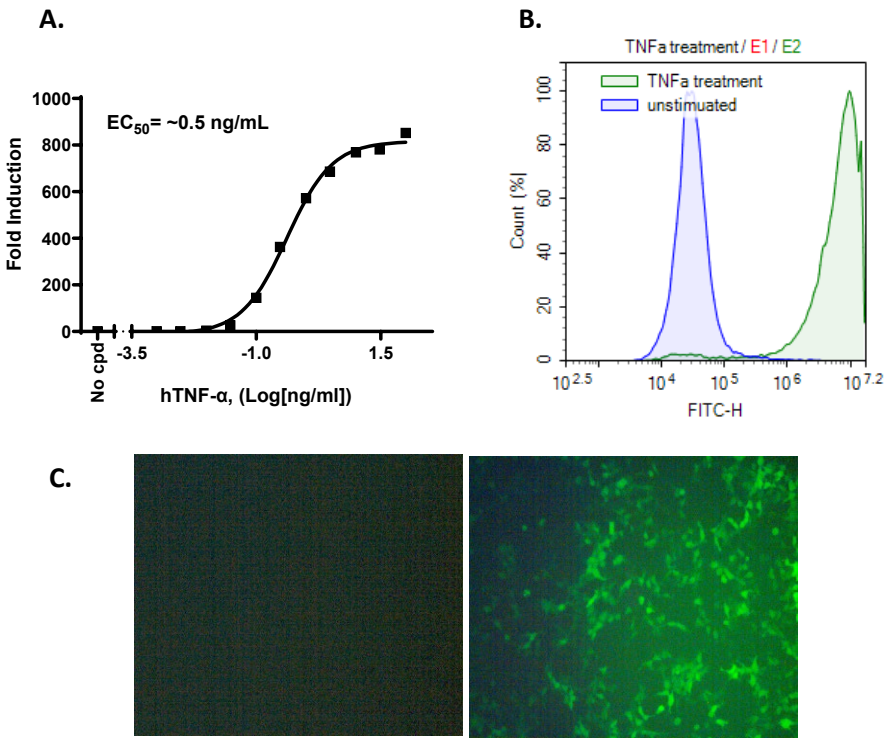

Figure 2: NF-κB Luciferase-eGFP reporter activity stimulated by human TNF-α in NF-κB Luciferase-eGFP Reporter HEK293 Cell Line.
A. NF-κB Luciferase-eGFP HEK293 cells were treated with increasing doses of human TNF-α for 5 hours and luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are shown as fold induction of luciferase reporter expression. B. NF-κB Luciferase-eGFP HEK293 cells were treated with 10 ng/ml of hTNF-α for 24 hours and eGFP expression was analyzed by flow cytometry. The x axis indicates the fluorophore intensity, while the y axis represents the % of cells. C. NF-κB Luciferase-eGFP HEK293 cells were treated with 10 ng/ml of hTNF-α for 24 hours and eGFP expression was analyzed by fluorescence microscopy. Left panel, untreated; right panel, TNF-α-treated cells.

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

References

Pessara U. and Koch N., 1990 *Mol Cell Biol*. 10(8): 4146-4154.
Baeuerle P.A., 1998 *Curr Biol*. 8(1): R19-R22.
Takada Y., *et al*., 2005 *J Biol Chem.* 280(17): 17203-17212.

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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*
NF- κB Reporter (Luc) HEK293 Cell Line	60650	2 vials
NF-κB TWO-Luciferase Reporter HEK293 Cell Line	82594	2 vials
NF-κB Luciferase Reporter Jurkat Cell Line	60651	2 vials
NF- κB Luciferase-eGFP Reporter Lentivirus	82336	500 µl x 2
NF- κB eGFP Reporter Lentivirus	79926	500 µl x 2
NF- κB Luciferase Reporter Lentivirus	79564	500 µl x 2

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