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

The BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 Cell Line is a HEK293 cell line that expresses full-length human TACI (transmembrane activator and CAML interactor, also known as Tumor necrosis factor receptor superfamily member 13B or CD267) (NM_012452.3) under the control of a CMV promoter, for high constitutive expression, and an inducible firefly luciferase reporter controlled by the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) Response Element located upstream of a minimal promoter. Upon ligand binding, TACI will initiate the NF-κB signaling pathway, leading to expression of the NF-κB-controlled luciferase reporter.

This cell line has been validated by flow cytometry and has been shown to respond to BAFF (B-cell activating factor) and APRIL (a proliferation-inducing ligand).

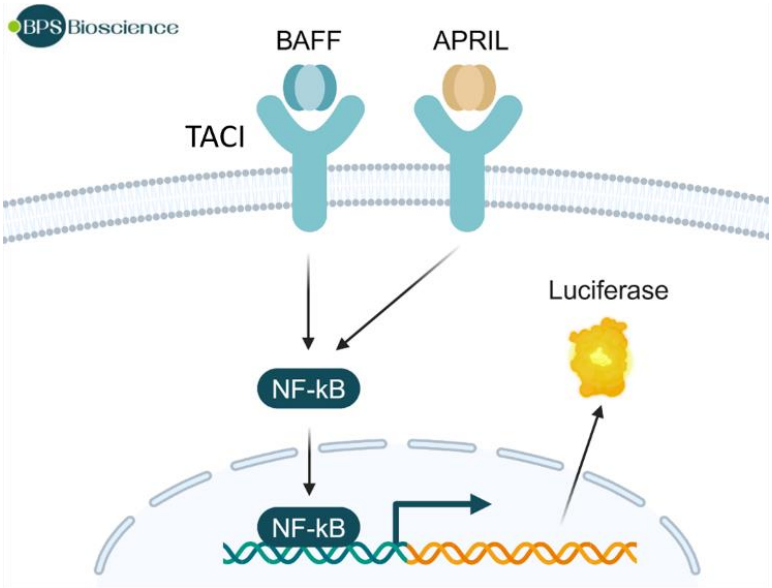

Figure 1: Illustration of the BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 Cell Line mechanism of action.

Background

TACI (transmembrane activator and CAML interactor), also known as Tumor necrosis factor receptor superfamily member 13B (TNFRSF13B) or CD267, is a type I membrane protein encoded by the TNFRSF13B gene. TACI belongs to the TNF receptor superfamily of proteins found in B cells and is a cell surface receptor for APRIL (A proliferation-inducing ligand, also known as TNFSF13) and BAFF (B-cell activating factor, also known as TNFSF13B, TALL1 or BLYS), binding both ligands with similar high affinity. TACI, BCMA (B-cell maturation antigen) and BAFFR are expressed at different stages of B cell development, with TACI being present in marginal zone, switched memory B cells and plasma cells. TACI is upregulated in activated B cells and is constitutively shed from the cell surface by ADAM10 (disintegrin and metalloprotease), resulting in a decoy receptor that is soluble. The levels of decoy receptor seem to correlate with some auto-immune disorders, such as SLE (systemic lupus erythematosus). Its role in auto-immune disorders led to the development of strategies around it, such as atacicept, a recombinant protein that blocks TACI-related signaling. High levels of TACI are also found in myeloma cells, and studies with CAR (chimeric antigen receptor)-T cells targeting TACI and BCMA, with the goal to bypass antigen escape when performing single antigen targeting, are underway for MM (multiple myeloma).

Application

- Monitor the TACI/NF-κB signaling pathway activity in a cellular model.
- Screen for compound activity on the TACI/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, 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 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 1G	BPS Bioscience #79544

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1 (Assay Medium)	BPS Bioscience #60187
BAFF, His-Avi-Tag Recombinant	BPS Bioscience #100194
APRIL, His-Avi-Tag Recombinant	BPS Bioscience #100254
BAFF/APRIL Dual Antagonist	BPS Bioscience #102254
Anti-BAFF Neutralizing Antibody	BPS Bioscience #102205
APC anti-human CD267 (TACI) Antibody	BioLegend #311911
96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate	
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, 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 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 Geneticin and 50 µg/ml Hygromycin B.

Assay Medium:
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. Incubate the cells with 0.05% Trypsin/EDTA for the minimal time required for cell detachment (about 30 seconds to 1 minute). Confirm cell detachment using a microscope.

3. Once the cells have detached, add Growth Medium 1G and transfer to a tube.

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

5. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:10 once or twice a week.

   Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells at a ~1:4 ratio at the beginning of culture. After several passages, the cell growth rate increases, and the cells can be split at a higher ratio.

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.

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

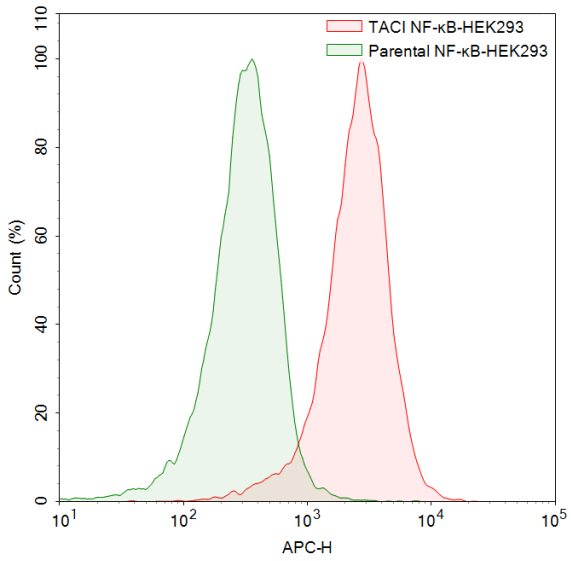

Figure 2: Flow cytometry analysis of TACI cell surface expression in BAFF/APRIL Responsive TACI-NF-κB HEK293 Cell Line.
BAFF/APRIL Responsive TACI-NF-κB HEK293 cells (red) and control parental NF-κB HEK293 cells (green) were stained with APC anti-human CD267 (TACI) Antibody (Biolegend #311911) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates APC intensity.

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 should include "Stimulated", "Unstimulated Control", and "Background Control" conditions.
- Assay B should include "BAFF + Antagonist", "No Antagonist Control", "BAFF, No Antagonist Control", and "Background Control" conditions.

A. Dose response of BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 Cell Line to BAFF and APRIL

1. Harvest BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 cells from culture and seed cells into the white clear-bottom 96-well microplate at a density of 30,000 cells per well in 100 µl of Thaw Medium 1. Leave a few wells empty for use as "Background Control".

2. Incubate cells at 37°C in a CO_2 incubator overnight.

3. Make a serial dilution of BAFF or APRIL in Thaw Medium 1 at 5-fold the desired final concentration (25 µl/well).

4. Gently add 25 µl of the diluted compounds to the "Stimulated" wells.

   Note: Cells can detach easily.

5. Add 25 µl of Thaw Medium 1 to the "Unstimulated Control" wells.

6. Add 125 µl of Thaw Medium 1 to the "Background Control" wells (for determining background luminescence).

7. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.

8. Add 125 µl of ONE-Step™ Luciferase reagent per well.

9. Rock at Room Temperature (RT) for ~15 minutes.

10. Measure luminescence using a luminometer.

11. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
The fold induction is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ Induction\ = \left(\frac{luminescence\ of\ Stimulated\ cells - background}{luminescence\ of\ Unstimulated\ \ cells - background}\right)$$

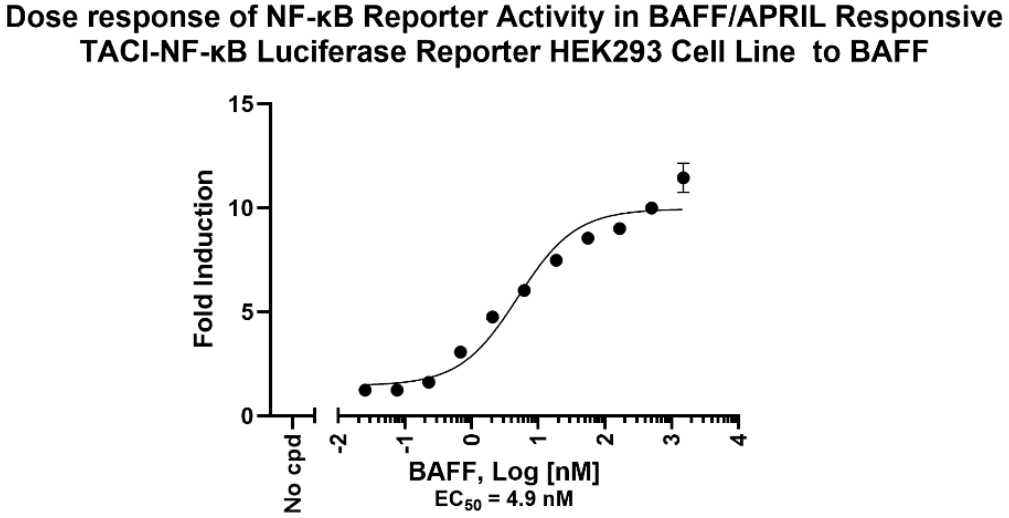

Figure 3. Dose response to BAFF of the BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 Cell Line measured as NF-κB-dependent luciferase reporter activity.
BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of BAFF for 6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. The results are shown as Fold Induction of NF-κB-dependent luciferase reporter activity.

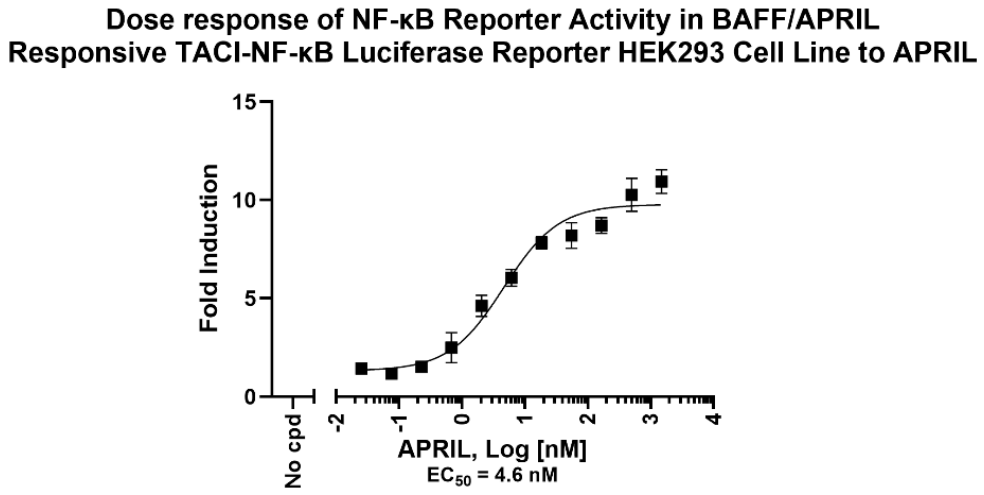

Figure 4. Dose response to APRIL of the BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 Cell Line measured as NF-κB-dependent luciferase reporter activity.
BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of APRIL for 6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. The results are shown as Fold Induction of NF-κB-dependent luciferase reporter activity.

B. Inhibition of BAFF-induced BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 Cell Line response by Anti-BAFF Neutralizing Antibodies, or BAFF/APRIL Dual Antagonist.

1. Harvest BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter HEK293 cells from culture and seed cells into the white clear-bottom 96-well microplate at a density of 30,000 cells per well in 100 µl of Thaw Medium 1. Leave a few of the wells empty for use as "Background Control".

2. Incubate cells at 37°C in a CO_2 incubator overnight.

3. Prepare a BAFF dilution in Thaw Medium 1 at 5x the final concentration to be tested (125 nM).

4. In a separate 96-well plate ("BAFF + Antagonist plate"), with no cells, make a serial dilution of Anti-BAFF Neutralizing Antibody or BAFF/APRIL Dual Antagonist diluted in Thaw Medium 1 containing 125 nM BAFF (prepared in step 3) at 5-fold the desired final concentration (25 µl/well).

5. Pre-incubate the "BAFF + Antagonist" plate at 37°C in a 5% CO_2 incubator for 40 minutes. Pipet solution up and down to ensure each well is fully mixed.

6. Transfer 25 µl of each well of the "BAFF + Antagonist" plate to the corresponding wells of the plate containing the cells. The final volume in each well will be 125 µl.

7. Add 25 µl of Thaw Medium 1 containing 125 nM BAFF (prepared in step 3) alone to the "No Antagonist Control" wells.

8. Add 25 µl of Thaw Medium 1 to the "No BAFF, No Antagonist Control" wells.

9. Add 125 µl of Thaw Medium 1 to "Background Control" wells (for determining background luminescence).

10. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.

11. Add 125 µl of ONE-Step™ Luciferase reagent per well.

12. Rock at RT for ~15 minutes.

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The Percentage is the average background-subtracted luminescence of "BAFF + Antagonist" treated wells divided by the average background-subtracted luminescence of "BAFF, No Antagonist Control" wells, multiplied by 100.

$$Percentage = \left(\frac{luminescence\ of\ "BAFF + Antagonist"\ treated\ cells - background}{luminescence\ of\ "BAFF, No\ Antagonist\ Control"\ cells - background}\right) \times 100$$

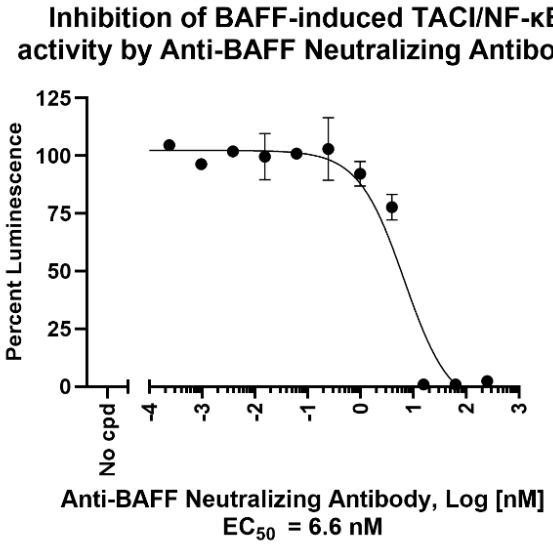

Figure 5: Inhibition of BAFF-induced TACI-NF-κB activity in BAFF/APRIL Responsive TACI-NF-κB Reporter HEK293 Cell Line by the Anti-BAFF Neutralizing Antibody.
Cells were treated with BAFF in the presence of increasing concentrations, or absence, of Anti-BAFF Neutralizing Antibody (#102205) for 6 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). Results are expressed as Fold Induction of "No BAFF, No Antagonist Control".

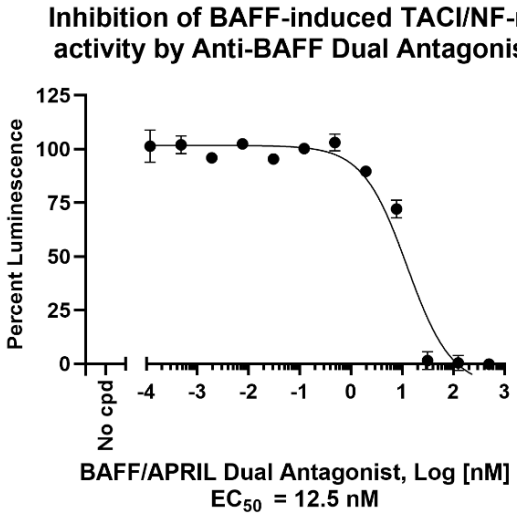

Figure 6: Inhibition of BAFF-induced TACI-NF-κB activity in BAFF/APRIL Responsive TACI-NF-κB Reporter HEK293 Cell Line by the BAFF/APRIL Dual Antagonist.
Cells were treated with BAFF in the presence of increasing concentrations, or absence, of BAFF/APRIL Dual Antagonist (#102254) for 6 hours. Luciferase activity was measured using ONE-Step™ luciferase reagent. Results are expressed as Fold Induction of "No BAFF, No Antagonist Control").

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

References

Smulski C., *et al.*, 2017 *Cell Reports* 18(9): P2189-2202.
Larson R., *et al.*, 2023 *Nature Communications* 14: 7509.

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*
TACI, Fc-Fusion (IgG1), Avi-Tag Recombinant	100283	100 µg
BAFF-R – CHO K1 Recombinant Cell Line (High, Medium or Low Expression)	79921	2 vials
BAFF:BAFFR [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	82536	96 reactions
BAFF/APRIL Responsive BCMA- NF-κB Luciferase Reporter Hek293 Cell Line	79755	2 vials
BAFF:APRIL [Biotinylated] Inhibitor Screening Assay Kit	79722	2 vials

Version 012825