Description

The BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 Cell Line is a HEK293 cell line that expresses full-length human BCMA (B-cell maturation antigen) (NM_001192) under the control of a CMV promoter, for high constitutive expression, and the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) – luciferase reporter. The firefly luciferase reporter is controlled by the NF-κB Response Element located upstream of a minimal promoter. Upon ligand binding, BCMA will initiate the NF-κB signaling pathway, leading to expression of the NF-κB-controlled luciferase reporter.

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

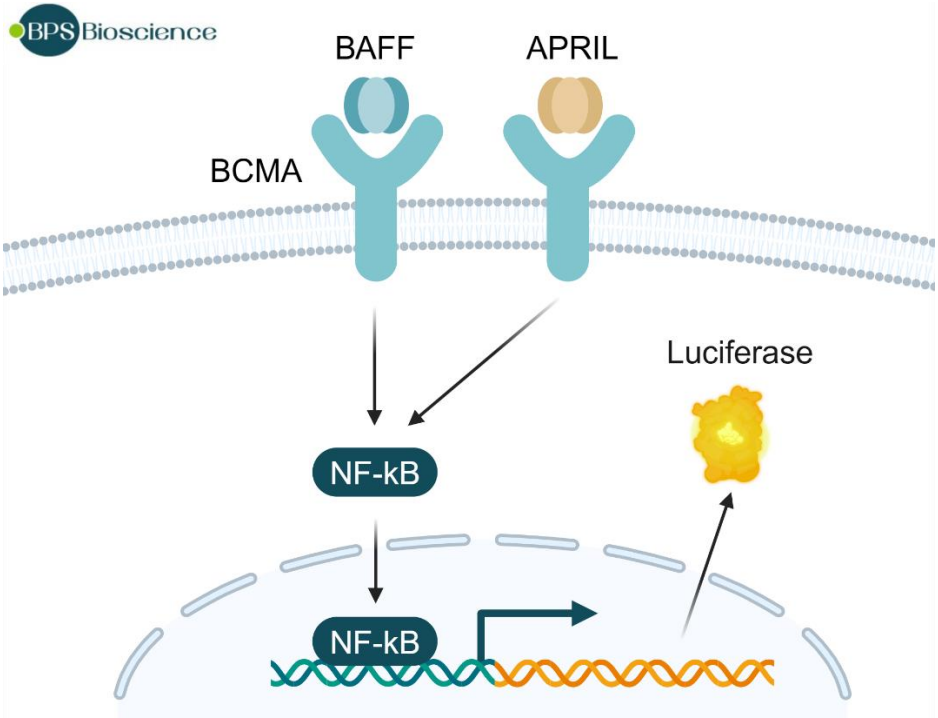

Figure 1: Illustration of the BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 Cell Line.

Background

B-Cell Maturation Antigen (BCMA), also known as tumor necrosis factor receptor superfamily member 17 (TNFRSF17) or CD269, is a type I membrane protein encoded by the TNFRSF17 gene. TNFRSF17 is a cell surface receptor of the TNF receptor superfamily, that recognizes its ligands including BAFF (B-cell activating factor) and APRIL (a proliferation inducing ligand). BCMA is preferentially expressed in mature B lymphocytes and also on Multiple Myeloma (MM) cells. In vitro, the activation of BCMA by its ligand promotes the differentiation and proliferation of B cells. A vast array of intracellular activity is involved in BCMA-induced signal transduction, including activation of the NF-κB signaling pathway. The bioactivity of BAFF and APRIL as soluble homotrimers distinguishes them from other TNFSF ligands such as TRAIL, FasL, and CD40L, which are only active as membrane-bound molecules.

Application

- Monitor the BCMA/NF-κB signaling pathway activity in a cellular model.
- Screen for compound activity on the BCMA/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 1A	BPS Bioscience #79528

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1A	BPS Bioscience #79528
Assay Medium 7B	BPS Bioscience #79718
BAFF, His-Avi-Tag Recombinant	BPS Bioscience #100194
APRIL, His-Avi-Tag Recombinant	BPS Bioscience #100254
Sibeprenlimab	MedChemExpress #HY-P99901
Anti-BAFF Neutralizing Antibody	BPS Bioscience #102205
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 does *not* contain selective antibiotics. However, Growth Media *does* 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 1A (BPS Bioscience #79528):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B and 400 µg/ml Geneticin®, G418 Sulfate.

Assay Medium 7B (BPS Bioscience #79718):
Opti-MEM I + 0.5% FBS + 1% Non-essential amino acids + 1 mM sodium pyruvate + 1% penicillin/streptomycin.

Cell Culture Protocol

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 1A.

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 1A 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 1A.

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 with ~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 1A 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

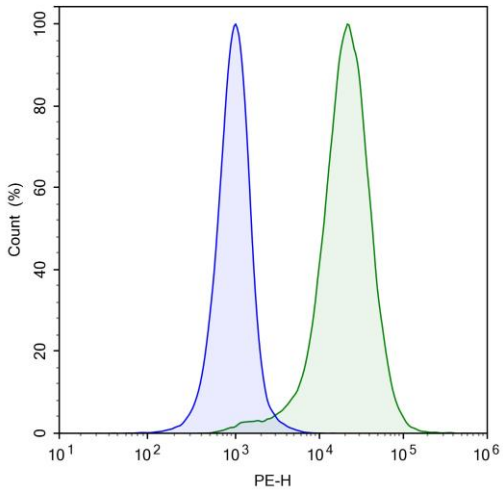

Figure 1: Flow cytometry analysis of BCMA cell surface expression in BAFF/APRIL Responsive BCMA NF-κB-HEK293 Cell Line.
BAFF/APRIL Responsive BCMA NF-κB-HEK293 cell (green) and control parental NF-κB-HEK293 (blue) cells were stained with PE anti-human CD269 (BCMA) Antibody (Biolegend #357503) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE 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", "Background Control" and "Unstimulated Control" conditions.
- Assay B should include "Background Control", "No Antibody Control", "No Antibody, No Agonist Control" and "Test Antibody" conditions.

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

1. Harvest BAFF/APRIL Responsive BCMA-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 a "Background Control".

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

3. Carefully remove the medium from the wells in order not to disturb the cells.

4. Add 50 µl of Assay Medium 7B to all wells.

5. Incubate the plate at 37°C in a CO_2 incubator for 20 to 24 hours.

6. Make a serial dilution of BAFF or APRIL in Assay Medium 7B at 2-fold the desired final concentration (50 µl/well).

7. Gently add 50 µl of the diluted compounds to the "Stimulated" wells.

   Note: Cells can detach easily.

8. Add 50 µl of Assay Medium 7B to the "Unstimulated Control" wells.

9. Add 100 µl of Assay Medium 7B to the "Background Control" wells (for determining background luminescence).

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

11. Add 100 µl of ONE-Step™ Luciferase reagent per well.

12. Rock at room temperature 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 fold induction is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

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

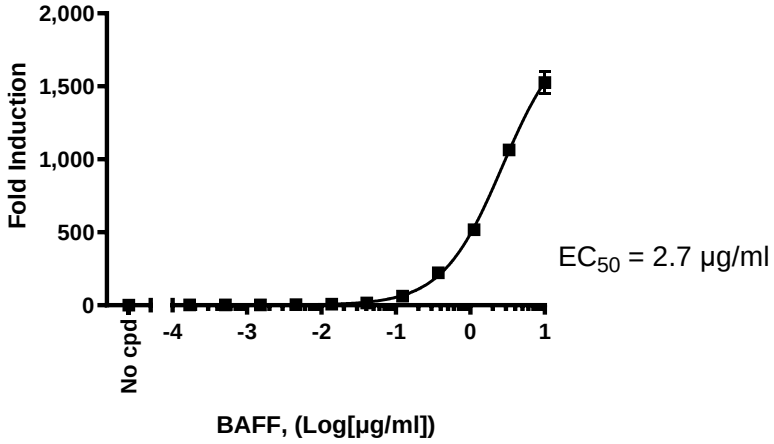

Figure 2. Dose response of NF-κB reporter activity in BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 Cell Line to BAFF.
BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of BAFF for 6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. The results are shown as Fold Induction of NF-κB reporter activity.

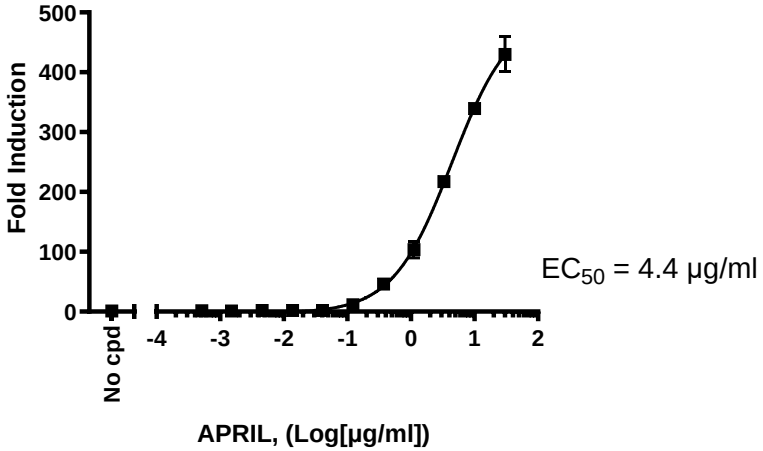

Figure 3. Dose response of NF-κB reporter activity in BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 Cell Line to APRIL.
BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of APRIL for 6 hours. Luciferase activity was measured with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

B. Inhibition of Agonist Induced BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 Cell Line response by Anti-BAFF and Anti-APRIL Antibodies

1. Harvest BAFF/APRIL Responsive BCMA-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 a "Background Control".

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

3. Carefully remove the medium from the wells in order not to disturb the cells.

4. Add 50 µl of Assay Medium 7B to all wells.

5. Incubate the plate at 37°C in a CO_2 incubator for 20 to 24 hours.

6. Make a serial dilution of anti-BAFF or APRIL antibodies in Assay Medium 7B at 4-fold the desired final concentration (25 µl/well).

7. Prepare a dilution of either APRIL or BAFF in Assay Medium 7B at concentrations 4-fold higher than the desired final concentrations (final [APRIL] on cells = 3 µg/ml and final [BAFF] on cells = 0.3 µg/ml) (25 µl/well).

8. In a separate 96-well plate (Antibody + Agonist plate), with no cells, prepare the following:

    a. Add 25 µl/well of the diluted test antibody to the "Test Antibody" wells.

    b. Add 25 µl/well of Assay Medium to the "No Antibody Control" wells.

    c. Add 50 µl/well of Assay Medium to the "No Antibody, No Agonist Control" wells.

    d. Add 25 µl/well of diluted agonist (APRIL or BAFF) to the "Test Antibody" and "No Antibody Control" wells.

9. Pre-incubate the Antibody + Agonist plate at 37°C in a 5% CO_2 incubator for 30 minutes. Pipet solution up and down to ensure each well is fully mixed.

10. Transfer 50 µl of each well of the Antibody + Agonist plate to the corresponding wells of the plate containing the cells. The final volume in each well will be 100 µl.

11. Add 100 µl of Assay Medium 7B to the "Background Control" wells (for determining background luminescence).

12. Incubate the plate at 37°C in a CO_2 incubator for 5 hours.

13. Add 100 µl of ONE-Step™ Luciferase reagent per well.

14. Rock at room temperature for ~15 minutes.

15. Measure luminescence using a luminometer.

16. 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 antibody treated wells divided by the average background-subtracted luminescence of "No Antibody" control wells.

$$Fold\ Induction\ = \left(\frac{luminescence\ of\ antibody\ treated\ cells - background}{luminescence\ of\ "No\ Antibody\ Control\ "\ cells - background}\right)$$

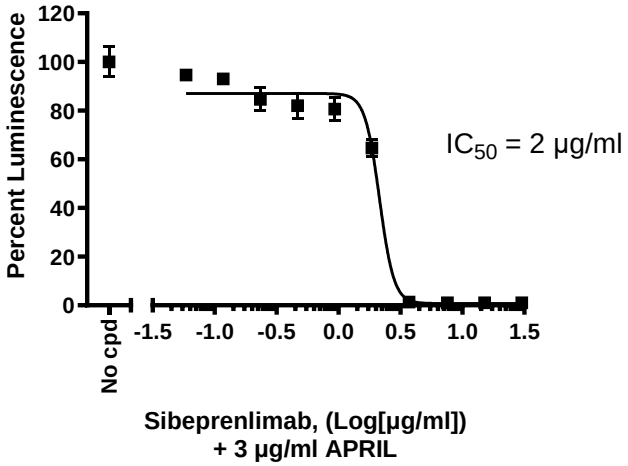

Figure 4: Inhibition of APRIL-induced BCMA/NF-κB activity by the anti-APRIL antibody, Sibeprenlimab, in the BAFF/APRIL Responsive BCMA/NF-κB Reporter HEK293 Cell Line.
Cells were treated with APRIL in the presence of increasing concentrations, or absence, of Sibeprenlimab for 5 hours. Luciferase activity was measured using ONE-Step™ luciferase reagent. Results are expressed as percent of control (in which luciferase activity in APRIL-stimulated cells without antibody was set at 100%).

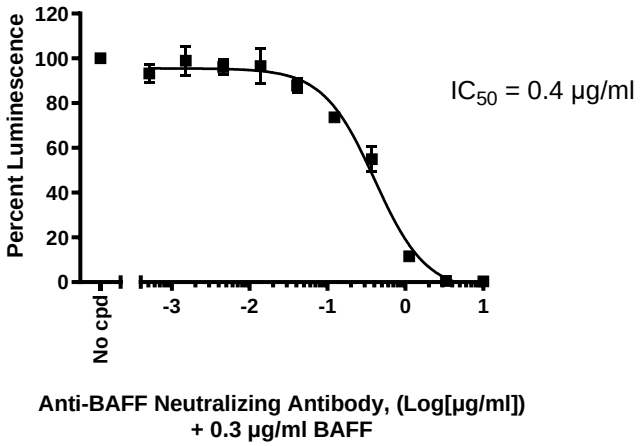

Figure 5: Inhibition of BAFF-induced BCMA/NF-κB activity by Anti-BAFF Neutralizing Antibody in the BAFF/APRIL Responsive BCMA/NF-κB Reporter HEK293 Cell Line.
Cells were treated with BAFF in the presence of increasing concentrations, or absence, of Anti-BAFF Neutralizing Antibody for 5 hours. Luciferase activity was measured using ONE-Step™ luciferase reagent. Results are expressed as percent of control (in which luciferase activity in BAFF-stimulated cells without antibody was set at 100%).

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

References

Hahne M., *et al.,* 1998 *J. Exp. Med*. 188:1185.
Yu G., *et al.,* 2000 *Nat. Immunol.* 1:252.
Yan M., *et al.,* 2000 *Nat. Immunol*. 1:37.
Gravestein L.A. and Borst J., 1998 *Sem. Immunol.* 10:423.

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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*
BCMA: APRIL[Biotinylated] Inhibitor Screening Assay Kit	79722	96 reactions
BAFFL: BCMA[Biotinylated] Inhibitor Screening Assay Kit	79667	96 reactions
BAFF-R-CHO K1 Recombinant Cell Line (High, Medium or Low Expression)	79921	2 vials
Human BAFF-R(CD268) Recombinant	90103	10 µg/50 µg
Anti-BCMA Antibody	101219	100 µg
BCMA Lentivirus	78714	500 µl x 2

Version 100124