Description

The BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line is a HEK293 cell line engineered to express a version of BAFFR (B-cell activating factor receptor, also known as TNFRSF13C, NM_052945.4] that is designed to couple with the canonical NF-kB (nuclear factor Kappa B) signaling pathway. The cells also express a firefly luciferase reporter driven by four copies of the NF-κB response element located upstream of the minimal TATA promoter. This cell line enables BAFF/BAFF-R signaling readout with a traditional luciferase reporter assay. Activation of the BAFF receptor in these cells can be monitored by measuring luciferase activity.

This cell line was validated in dose-response assays using BAFF ligand, and a BAFF receptor targeting antibody Ianalumab. It was also validated with two BAFF blockers: anti-BAFF neutralizing antibody and the Dual BAFF/APRIL Antagonist, Povetacicept.

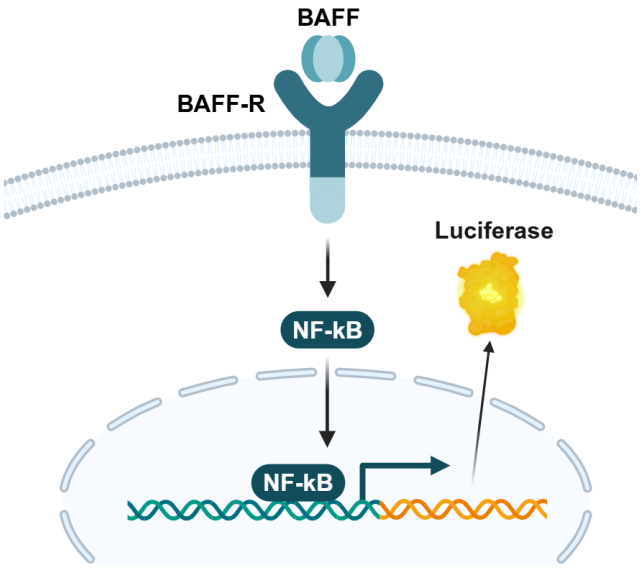

Figure 1: Illustration of the mechanism by which the luciferase reporter is activated in response to BAFF binding to its receptor.

Background

BAFF (B-cell activating factor), also known as TNFSF13B, is a member of the TNF (Tumor Necrosis Factor) super family that is produced by innate immune cells, where it undergoes furin-mediated cleavage and is released as a soluble homotrimer. BAFF influences B-cell dynamics through its interaction with three B-cell expressed receptors: BAFF-R, TACI (Transmembrane activator and CAML interactor) and BCMA (B-cell maturation antigen). While TACI and BCMA receptors also bind to the TNF super family member APRIL (A proliferation-inducing ligand), BAFF-R interacts only with BAFF ligand. Downstream signaling from BAFF/BAFF-R promotes B cell maturation and survival and has been shown to play a role in autoimmune diseases. Unique among the BAFF/APRIL family of receptors, BAFF-R preferentially signals through the non-canonical NF-κB pathway, making it challenging to study with existing reporter systems. Therapies targeting BAFF ligand and BAFF receptor are currently under development to treat a variety of B cell mediated autoimmune conditions including IgA nephropathy, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

Application

- Screen BAFF Receptor-targeting antibodies.
- Screen BAFF ligand-neutralizing therapies.

Note: Since the cell line has been engineered to force signaling through the canonical NF-kB signaling pathway, it is not suitable for studying the endogenous intracellular signaling downstream of BAFF-R.

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 19B	BPS Bioscience #83761

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium 1F	BPS Bioscience #84189
Ianalumab	BPS Bioscience #83597
Anti-BAFF Neutralizing Antibody	BPS Bioscience #102205
BAFF/APRIL Dual Antagonist (Povetacicept)	BPS Bioscience #102254
BAFF, His-Avi-Tag Recombinant	BPS Bioscience # 100194
FluoSite Anti-BAFF-R Antibody, FITC-Labeled	BPS Bioscience # 102988
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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 19B (BPS Bioscience #83761):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 50 µg/ml Hygromycin and 0.5 µg/ml Puromycin.

Media Required for Functional Cellular Assay

Assay Medium 1F (BPS Bioscience # 84189):

Opti-MEM with 0.5% FBS plus 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 19B.

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 19B 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 19B.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:8 once 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 19B 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 aliquots into cryogenic vials. 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.

Validation Data

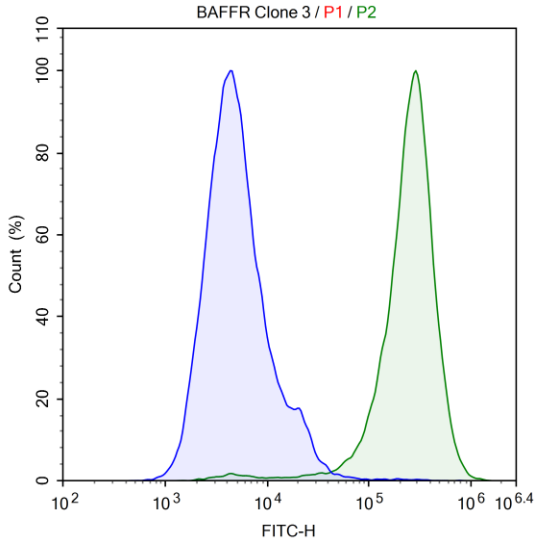

Figure 2: Flow cytometry analysis of BAFF-R cell surface expression in BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line.
BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line (green) or control NF-κB - HEK293 cells (blue) were stained with FluoSite Anti-BAFF-R Antibody, FITC-Labeled (#102988) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Functional characterization of BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The assay should be performed in triplicate.

Assay Medium: Assay Medium 1F (BPS Bioscience #84189)

A. Dose response of BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line to BAFF

- This experiment measures the effect of an agonist on reporter activation.
- The assay should include “Stimulated”, “Unstimulated” (negative control, no agonist) and “Background Luminescence” (no cells) conditions.

1. Seed the cells into a white clear-bottom 96-well cell culture plate at a density of ~30,000 cells per well in 90 µl of Assay Medium. Leave a few wells empty for use as the cell-free control wells (“Background Luminescence”).

2. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

3. The next day, prepare a serial dilution of BAFF in Assay Medium at concentrations 10-fold higher than the desired final concentration (10 µl/well).

4. Add 10 µl of BAFF ligand dilutions to the “Stimulated” wells.

5. Add 10 µl of Assay Medium to the "Unstimulated" wells.

6. Add 100 µl of Assay Medium to the "Background Luminescence" wells (for determining background luminescence).

7. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

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

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

10. Measure luminescence using a luminometer.

11. Data analysis: The fold induction of the NF-κB luciferase reporter is the average luminescence of the stimulated wells divided by the average luminescence of the unstimulated control wells.

Note: Luminescence values of unstimulated cells are typically low in this cell line and can be comparable to the cell-free media controls. For this reason, we do not recommend performing background subtraction as this can result in negative values and distortions of the fold induction.

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells}{average\ luminesence\ of\ unstimulated\ cells}$$

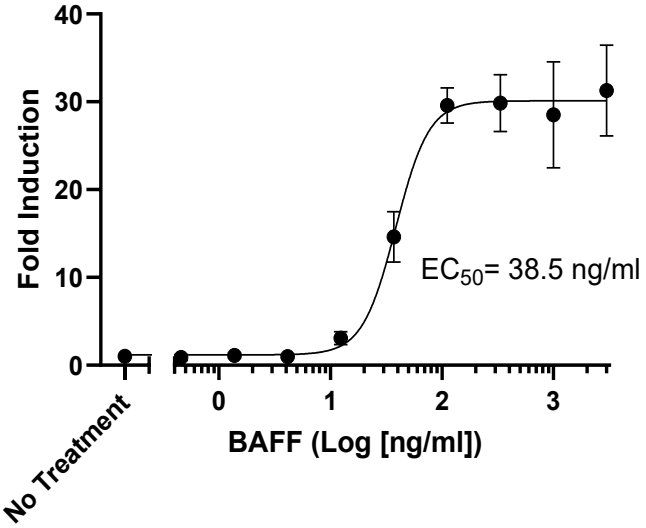

Figure 3: Dose response of BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line to human BAFF.
BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 cells were treated with increasing concentrations of human BAFF for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

B. Dose response of BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line to BAFF-R targeting antibodies.

- This experiment measures the effect of BAFF Receptor targeting antibodies against stimulation by BAFF.
- The assay should include "Stimulated, No Compound", "Unstimulated, No Compound", "Background Luminescence" and "Stimulated, Test Compound" conditions.

1. Seed the cells into a white clear-bottom 96-well cell culture plate at a density of ~30,000 cells per well in 80 µl of Assay Medium. Leave a few wells empty for use as the cell-free control wells ("Background Luminescence").

2. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

3. The next day, prepare a serial dilution of the BAFF-R targeting antibody in Assay Medium at concentrations 10-fold higher than the desired final concentration (10 µl/well).

4. Add 10 µl of BAFF-R targeting antibody dilutions to the "Stimulated, Test Compound" wells.

5. Add 10 µl of Assay Medium to the "Unstimulated, No Compound" and "Stimulated, No Compound" wells.

6. Incubate at 37°C with 5% CO_2 for 30 to 60 minutes.

7. Prepare a 3 µg/ml solution of human BAFF in Assay Medium (10-fold the final desired concentration of 300 ng/ml) (10 µl/well).

8. Add 10 µl of diluted human BAFF to the "Stimulated, Test Compound" and "Stimulated, No Compound" wells.

9. Add 10 µl of Assay Medium to the "Unstimulated, No Compound" wells.

10. Add 100 µl of Assay Medium to the "Background Luminescence" wells (for determining background luminescence).

11. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

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

13. Rock gently at RT for ~15 minutes.

14. Measure luminescence using a luminometer.

15. Data analysis: The percent activity of the NF-κB luciferase reporter is the luminescence of the compound-treated, stimulated cells divided by the average luminescence of the stimulated control wells.

    Note: Luminescence values of unstimulated cells are typically low in this cell line and can be comparable to the cell-free media controls. For this reason, we do not recommend performing background subtraction as this can result in negative values and distortions of the fold induction.

$$Percent\ Activity = \frac{Luninescence\ of\ compound\ treated, stimulated\ cells}{Luminescence\ of\ no\ compound, stimulated\ cells}$$

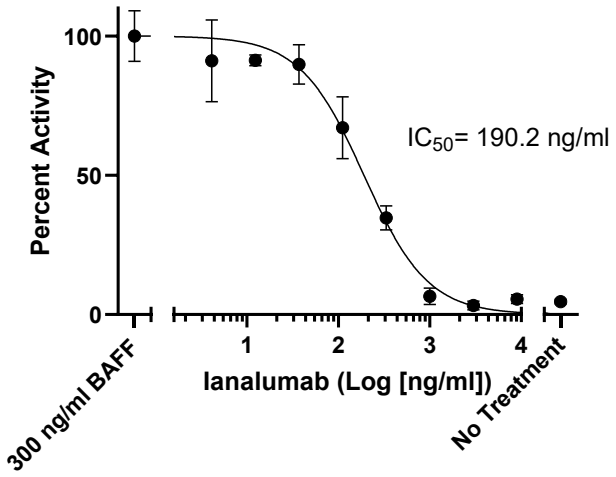

Figure 4: Dose response of BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line to Ianalumab.
BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 cells were incubated with increasing concentrations of the anti-BAFF-R antibody Ianalumab (#83597) for 30 minutes before stimulation with 300 ng/ml of BAFF. After a 5-hour incubation, luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of NF-κB reporter activity (compared to cells stimulated by BAFF in the absence of antibody, set at 100%).

C. Dose response of BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line to BAFF-targeting agents.

- This experiment measures the effect of BAFF targeting agents, such as neutralizing antibodies against stimulation by BAFF.
- The assay should include "Stimulated, No Compound", "Unstimulated, No Compound", "Background Luminescence" and "Stimulated, Test Compound" conditions.

1. Seed the cells into a white clear-bottom 96-well cell culture plate at a density of ~30,000 cells per well in 80 µl of Assay Medium. Leave a few wells empty for use as the cell-free control wells ("Background Control").

2. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

3. The next day, prepare a serial dilution of the BAFF-targeting antibody in Assay Medium at concentrations 10-fold higher than the desired final concentration (10 µl/well).

4. Prepare at 3 µg/ml solution of human BAFF in Assay Medium (10-fold the final desired concentration of 300 ng/ml) (10 µl/well).

5. Prepare a separate 96 well plate for pre-incubations. Add 10 µl/well of each dilution of BAFF-targeting agent prepared in step 3 and 10 µl/well of BAFF prepared in step 4. This is the BAFF/Inhibitor Mix. Pre-incubate at RT for 30-60 minutes.

6. Add 20 µl of BAFF/Inhibitor Mix dilution to the "Stimulated, Test Compound" wells.

7. Add 10 µl of 3 µg/ml BAFF solution only and 10 µl pf Assay Medium to the "Stimulated, No Compound" wells.

8. Add 20 µl of Assay Medium to the "Unstimulated, No Compound" wells.

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

10. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

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

12. Rock gently at RT for ~15 minutes.

13. Measure luminescence using a luminometer.

14. Data analysis: The percent activity of the NF-κB luciferase reporter is the luminescence of the compound-treated, stimulated cells divided by the average luminescence of the stimulated control wells.

    Note: Luminescence values of unstimulated cells are typically low in this cell line and can be comparable to the cell-free media controls. For this reason, we do not recommend performing background subtraction as this can result in negative values and distortions of the fold induction.

$$Percent\ Activity = \frac{Luninescence\ of\ compound\ treated, stimulated\ cells}{Luminescence\ of\ no\ compound, stimulated\ cells}$$

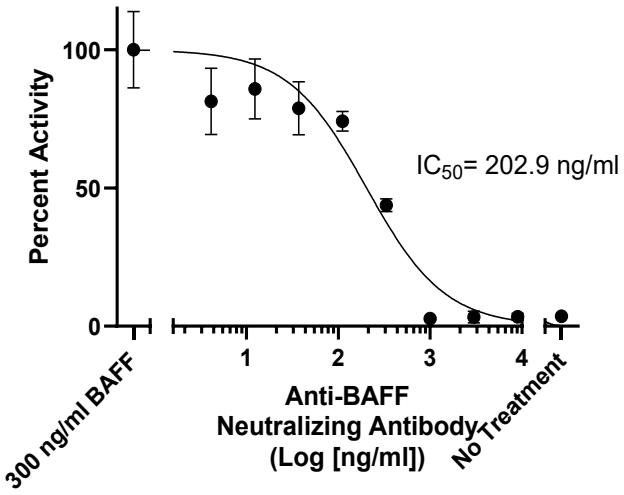

Figure 5: Dose response of BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line to Anti-BAFF Neutralizing Antibody.

A serial dilution of Anti-BAFF Neutralizing Antibody (#102205) was prepared and preincubated with BAFF for 30 minutes. After the 30-minute preincubation, the BAFF/Antibody Mix was added to the cells. After a 5-hour incubation, luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of NF-κB reporter activity (compared to cells stimulated by BAFF in the absence of antibody set at 100%).

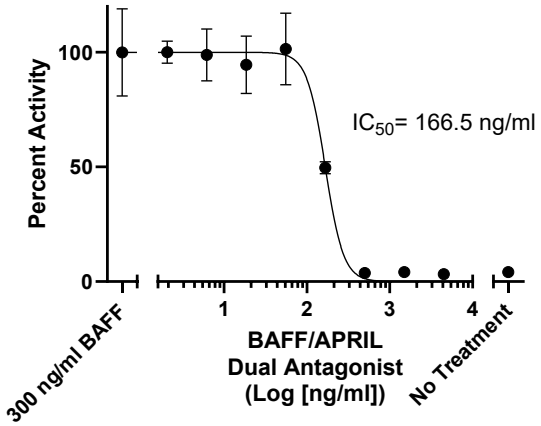

Figure 6: Dose response of BAFF Responsive BAFF-R NF-kB Luciferase Reporter HEK293 Cell Line to the BAFF/APRIL Dual Antagonist (Povetacicept).

A serial dilution of BAFF/APRIL Dual Antagonist (Povetacicept) (#102254) was prepared and preincubated with BAFF for 30 minutes. After the 30-minute preincubation, the BAFF/Antibody Mix was added to the cells. After a 5-hour incubation, luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of NFκB reporter activity (compared to cells stimulated by BAFF in the absence of antibody set at 100%).

Data shown is representative.

References

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