6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

BCMA / NF-κB – Reporter HEK293 Recombinant Cell Line
Catalog #: 79755

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.

Description

The BCMA / NF-κB - HEK293 recombinant cell line has been transfected with full-length human BCMA cDNA (Genbank #NM_001192) under control of a CMV promoter for high constitutive expression. The NF-κB–luciferase reporter is also stably integrated into the genome. The firefly luciferase gene is controlled by four copies of the NF-κB Response Element upstream of a minimal promoter. Upon ligand binding, active BCMA will initiate the NF-κB signaling pathway, leading to expression of the NF-κB-controlled luciferase reporter.

Applications

- Monitor the BCMA/NF-κB signaling pathway activity.
- Screen for activators or inhibitors of the BCMA/NF-κB signaling pathway.

Format
Two vials containing ~ 2 x 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage
Store in liquid nitrogen immediately upon receipt. Do not store long-term at -80°C or on dry ice.

Mycoplasma Testing
This cell line has been screened using the Venor™ GeM Mycoplasma Detection Kit, PCR Based (Sigma, #MP0025) to confirm the absence of Mycoplasma contamination.

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Culture Medium:

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 1A (BPS Bioscience, #79528): Thaw Medium 1, 400 µg/ml of Geneticin (Thermo Fisher, #11811031) and 100 µg/ml Hygromycin B (Thermo Fisher, #10687010).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1A.

Recommended Culture Condition:

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 **(no Geneticin or Hygromycin)**, spin down cells at 1000 rpm, and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 **(no Geneticin or Hygromycin)**.Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 1 **(no Geneticin or Hygromycin)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 1A **(contains Geneticin and Hygromycin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS) and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1A **(contains Geneticin and Hygromycin)** and transfer to a tube. Spin down cells, resuspend cells in Growth Medium 1A **(contains Geneticin and Hygromycin)** and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:6 to 1:10 weekly 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 culturing. After several passages, the cell growth rate increases and the cells can be split with a higher ratio.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (no Geneticin or Hygromycin) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.

It is recommended to expand the cells and freeze down more than 10 vials of cells at an early passage for future use.

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Functional Validation and Assay Performance
The following assays are designed for a 96-well plate format. To perform assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

Materials Required but Not Supplied

- Thaw Medium 1 (BPS Bioscience, #60187)
- Growth Medium 1A (BPS Bioscience #79528)
- **Assay Medium 7B (BPS Bioscience, #79718):**
  - Opti-MEM I (Life Technologies #31985-062) supplemented with 0.5% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).
- Recombinant human BAFF (BPS Bioscience, #100194)
- Recombinant human APRIL (BPS Bioscience, #100254)
- 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

Transfection and Assay Protocol – Ligand dose response

1. Harvest BCMA / NF-κB Reporter – HEK293 cells from culture in growth medium 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 cell-free control.

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

2. The next day, carefully remove the medium from wells. Add 50 µl of Assay Medium 7B (Opti-MEM with 0.5% serum) to wells.

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

3. Make a serial dilution of BAFF or APRIL in 50 µl of Assay Medium 7B, keep the same amount of PBS in each dilution.

   Gently add compounds to wells. Cells can detach easily.
   Add 50 µl of assay medium to the unstimulated control wells.
   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

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

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4. Prepare luciferase reagents using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
   The fold induction for each treatment concentration = average background-subtracted luminescence of stimulated wells / average background-subtracted luminescence of unstimulated control wells.

Figure 1a. Dose response of NF-κB reporter activity to BAFF in the presence of 0.5% FBS. The results are shown as Relative Light Units of NF-κB reporter activity.
The EC50 of BAFF is ~ 277 ng/ml.

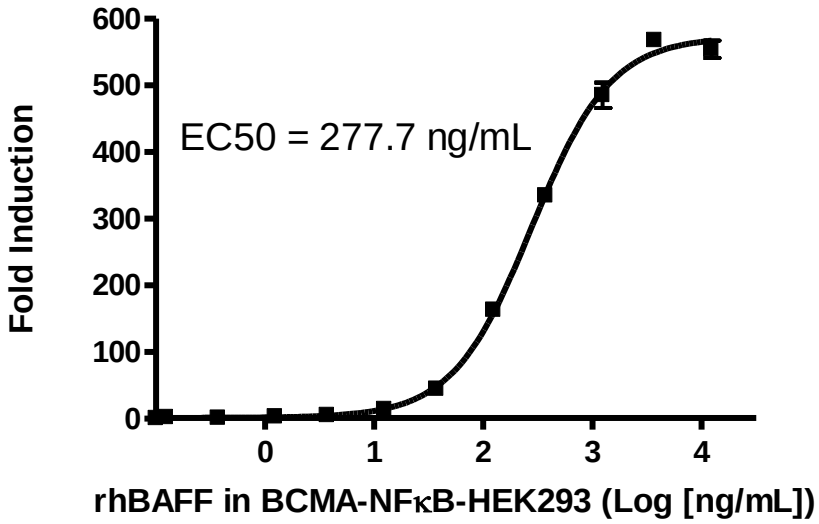

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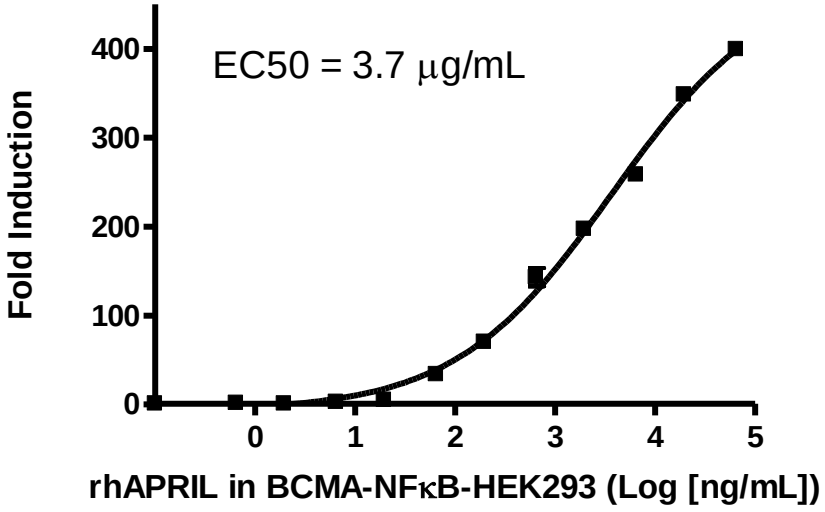
Figure 1b. Dose response of NF-κB reporter activity to APRIL in the presence of 0.5% FBS.
The results are shown as Relative Light Units of NF-κB reporter activity. The EC50 of APRIL is ~ 3.7 µg/ml.



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References

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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.

Related Products

Product Name	Catalog #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 mL
ONE-Step™ Luciferase Assay System	60690-2	100 mL
ONE-Step™ Luciferase Assay System	60690-3	1 L
Thaw Medium 1	60187-1	100 mL
Thaw Medium 1	60187-2	500 mL
Growth Medium 1A	79528	500 mL
Assay Medium 7B	79718-1	100 mL
Assay Medium 7B	79718-2	500 mL
BAFF, His-Avi-Tag HiP™	100194	50 µg
APRIL, His-Avi-Tag	100254	100 µg

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