Description

Recombinant clonal stable CHO cell line constitutively expressing full length human BCMA protein, also known as TNFRSF17, (Genbank #NM_001192). Surface expression of BCMA was confirmed by flow cytometry. Each stable clonal cell line was selected for different levels of BCMA expression (High, Medium, Low) to mimic different stages of cancer target cells with various BCMA expression levels.

Background

B-cell maturation antigen (BCMA), also known as CD269 or tumor necrosis factor receptor superfamily member 17 (TNFRSF17), is a cell surface receptor of the TNF receptor superfamily that recognizes B-cell activating factor (BAFF). BCMA is preferentially expressed in mature B lymphocytes and also on Multiple Myeloma (MM) cells. BCMA is a highly attractive target antigen for immunotherapy because of its restricted expression in non-malignant tissue but almost universal expression on MM cells. CART-BCMA is an autologous T cell product engineered by lentiviral transduction to express a fully human BCMA-specific CAR (chimeric antigen receptor). CAR T cells targeting BCMA have pre-clinical anti-MM activity, and in 2017, the FDA granted BCMA CAR T-Cell immunotherapy breakthrough designation in Multiple Myeloma.

Application

1. Useful as BCMA-expressing target cells in co-culture assay with BCMA-CAR-T cell, for both BCMA-specific cell killing assay and cytokine production assay.
2. Useful for screening and validating antibodies against BCMA and anti-BCMA CAR-T for immunotherapy research and drug discovery.
3. Useful for BCMA binding assays to screen for BCMA ligands.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience, #79796)

Parental Cell Line

CHO-K1 cells, Chinese Hamster Ovary, 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 3	BPS Bioscience #60186
Growth Medium 3D	BPS Bioscience #79539

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Materials Required for Cellular Assay

Name	Ordering Information
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	BPS Bioscience #60621
Thaw Medium 2	BPS Bioscience #60184
Anti-BCMA/Anti-CD3 Bispecific Antibody	BPS Bioscience #100689
96-well tissue culture-treated white clear-bottom assay plate	
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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience, #60186):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3D (BPS Bioscience, #79539):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience, #60184):
RPMI1640 medium supplemented with 10% FBS, 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 3 **(no Geneticin)**.
   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 3 **(no Geneticin)**.

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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 3 **(no Geneticin)**, 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 3D **(contains Geneticin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3D and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3D **(contains Geneticin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:6 to 1:8 weekly or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3D 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) 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 storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

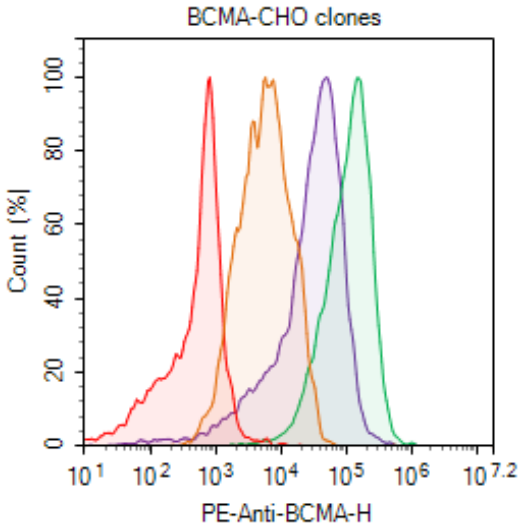

Figure 1. Expression of BCMA validated by flow cytometry. Flow cytometry using PE-conjugated anti-human BCMA (CD269) antibody (Biolegend, #357504) detects BCMA surface expression of BCMA-CHO Recombinant Cell Lines with different expression levels: #79500-H, high expresser: green; #79500-M, medium expresser: purple; #79500-L, low expresser: brown; WT CHO negative control: red.

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B. Cellular Assay Protocol

1. Harvest BCMA CHO cells from culture and seed cells at a density of 30,000 cells per well into a white clear-bottom 96-well microplate in 50 µl of assay medium (Thaw Medium 2). Incubate cells at 37° in a CO_2 incubator for several hours to allow them to attach.

2. Prepare a serial dilution of anti-BCMA/anti-CD3 antibody in assay medium in a fresh 96-well plate. The concentration of antibody here is 4x the final treatment concentration. Set up each treatment in at least triplicate. Add 25 µl of each dilution to the BCMA CHO cells.

3. Harvest the NFAT-reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute cells to $1.2x10^6$ / ml in assay medium. The final cell density of NFAT Reporter- Jurkat cells is 30,000 cells per well. Add 25 µl of NFAT-reporter-Jurkat cells to the BCMA CHO cells. The final volume will be 100 µl per well including both cell lines and the test antibody.

4. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence). Incubate the plates at 37° in a CO_2 incubator overnight.

5. The next day, perform the luciferase assay using the **ONE-Step™ Luciferase Assay System**. Add 100 µl of **ONE-Step™** Luciferase reagent per well and rock gently at room temperature for ~15 minutes. Measure luminescence using a luminometer. *If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.*

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NFAT luciferase reporter expression = background-subtracted luminescence of treated well / average background-subtracted luminescence of untreated control wells.

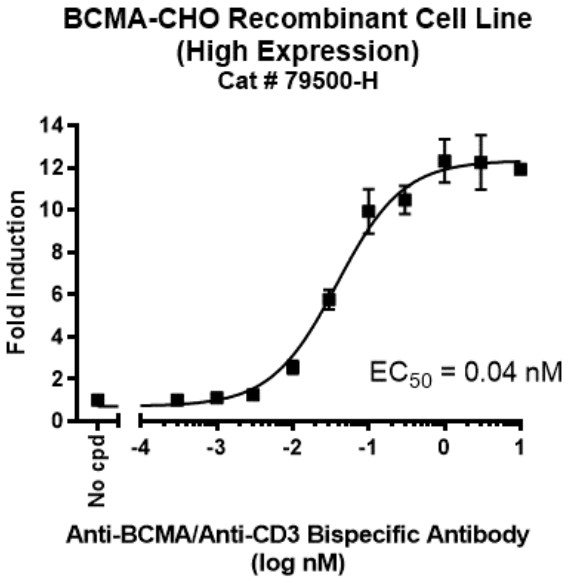

Figure 2. Testing an anti-BCMA/anti-CD3 bispecific antibody. Dose response of a bispecific antibody in NFAT Reporter-Jurkat cells co-cultured with BCMA CHO (high expressing) cells.

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Sequence
Human BCMA (accession number NM_001192) was cloned into pIRESneo3.

MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNAILWTCLGLSLIISLAVFVLMFLLRKINSEPLKD
EFKNTGSGLLGMANIDLEKSRTGDEIILPRGLEYTVEECTCEDCIKSKPKVDSDHCFPLPAMEEGATILVTTKTNDYCKSLPAALSAT
EIEKSISAR

References

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   Leuk Lymphoma. 2017 Nov; **6**:1-12
2. Sanchez E, *et al.* The clinical significance of B-cell maturation antigen as a
   therapeutic target and biomarker.
   Expert Rev Mol Diagn. 2018 Mar; **7**:1-11.
3. Sohail A., *et al.* Emerging immune targets for the treatment of multiple myeloma.
   Immunotherapy. 2018 Feb 1; **10(4)**:265-282.
4. Sidaway P., *et al.* Anti-BCMA CAR T cells show promise in MM.
   Nat Rev Clin Oncol. 2016 Sep;**13(9)**:530.

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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 CHO Recombinant Cell Line (High or Low Expression)	79500	2 vials
BCMA, Fc-Fusion, Avi-Tag	79465	100 µg
BCMA, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled	79467	50 µg
BAFF	90100	10 µg/100 µg/1 mg
Human BAFF-R(CD268)	90103	10 µg/50 µg

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