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

- Co-culture assays with BCMA-CAR-T cell, for both BCMA-specific cell killing assay and cytokine production assessment.
- Screening and validating antibodies against BCMA and anti-BCMA CAR-T for immunotherapy research and drug discovery.
- BCMA binding assays in order to identify BCMA ligands.

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

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

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	
PE-conjugated anti-human BCMA (CD269) antibody	Biolegend #357504
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℃ 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 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. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 3 to the conical tube containing the cells. Thaw Medium 3 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell viability. Change medium to fresh Thaw Medium 3 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3D.

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 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3D 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 3D. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 weekly 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 0.25% 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 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.

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.

A. Validation Data

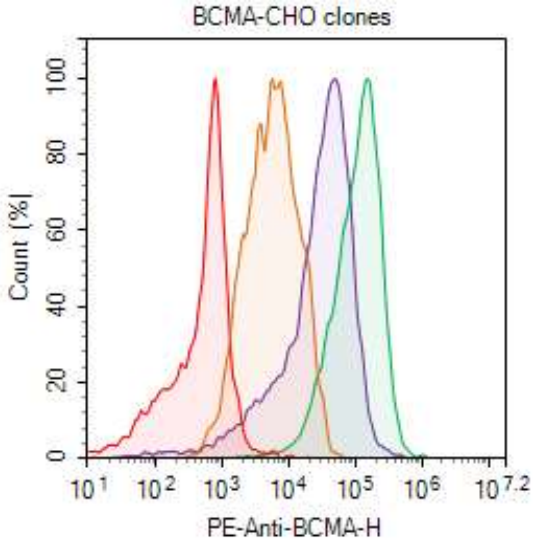

Figure 1. Flow cytometry analysis of BCMA expression in BCMA CHO Cell Line.
Flow cytometry analysis with PE-conjugated anti-human BCMA (CD269) antibody detected BCMA surface expression in the BCMA-CHO Recombinant Cell Lines with different expression levels: #79500-H, high expresser: green; #79500-M, medium expresser: purple; #79500-L, low expresser: orange; WT CHO negative control: red.

B. Cellular Assay Protocol

- The following assays are designed for 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.
- The assay should be performed in triplicate.
- The assay should include cell-free wells and NFAT Reporter (Luc)– Jurkat Recombinant Cells as controls.

1. Seed cells at a density of 30,000 cells per well in 50 µl of Assay Medium (Thaw Medium 2) into a white clear-bottom 96-well microplate.

2. Incubate cells at 37°C in a CO_2 incubator for several hours to allow them to attach.

3. Prepare a serial dilution of anti-BCMA/anti-CD3 antibody at 4x the final treatment concentration in Assay Medium in a second 96-well plate.

4. Add 25 µl of each antibody dilution to the BCMA CHO cells.

5. Dilute NFAT Reporter (Luc)– Jurkat cells to $1.2x10^6$ / ml in Assay Medium.

6. Add 25 µl of diluted NFAT Reporter (Luc)– Jurkat cells to each well with BCMA CHO cells.

7. Add 100 µl of Assay Medium to cell-free control wells (for determining background luminescence).

8. Incubate the plates at 37°C in a CO_2 incubator overnight.

9. The next day, add 100 µl of ONE-Step™ Luciferase reagent per well.

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

11. Measure luminescence using a luminometer.

12. 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.

$$Fold\ induction = \frac{average\ Lum\ sample - average\ backgound}{average\ Lum\ control - average\ background}$$

Sequence
Human BCMA (NM_001192) was cloned into pIRESneo3.

MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNAILWTCLGLSLIISLAVFVLMFLLRKINSEPLKD
EFKNTGSGLLGMANIDLEKSRTGDEIILPRGLEYTVEECTCEDCIKSKPKVDSDHCFPLPAMEEGATILVTTKTNDYCKSLPAALSAT
EIEKSISAR

References

1. Ghosh A., *et al.* 2017 *Leuk Lymphoma.* 6:1-12
2. Sanchez E., *et al.*, 2018 *Expert Rev Mol Diagn.* 7:1-11.
3. Sohail A., *et al.*, 2018 *Immunotherapy.* 10(4):265-282.
4. Sidaway P., *et al.* 2016 *Nat Rev Clin Oncol.* 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*
Anti-BCMA CAR/NFAT (Luciferase) Reporter Jurkat Cell Line	79694	2 vials
BCMA Lentivirus	78714	500 µl x 2
BCMA, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled	79467	50 µg
BAFF Recombinant	90100	10 µg/1 mg
Human BAFF-R(CD268) Recombinant	90103	10 µg/50 µg