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# Data Sheet

## Anti-BCMA CAR Jurkat/NFAT (Luciferase) Reporter Cell Line

**Catalog #: 79694**

### Product Description

The anti-BCMA CAR Jurkat/NFAT-luciferase reporter cell line is a stable cell line made from the anti-BCMA scFV CAR lentivirus (BPS Bioscience #79701). It has been validated for anti BCMA-CAR expression by FACS, and for luciferase reporter activation stimulated by both soluble BCMA protein (BPS Bioscience #79467) and BCMA/CHO target cells (BPS Bioscience #79500).

### Background

The development of CAR-T cells is a complex process that requires I) screening and sequencing of mAbs that are specific to the cancer antigens; II) synthesis of scFv cDNA and clone into Chimeric Antigen Receptor (CAR) cassette in Lentivector (e.g. anti-BCMA scFv in 3rd generation CAR cassette in lentivector); III) packaging and production of high titer lentivirus CAR encoding lentivirus; IV) isolation, activation and expansion of patient-derived T cells that exhibit a specific cellular phenotype (e.g. CD4+ or CD8+ or a mix); V) and transduction of activated T cells with CAR-encoding lentivirus; VI) Validation of engineered CAR-T cells through FACS and functional analysis. BPS has developed an anti-BCMA CAR Jurkat/NFAT-luciferase stable reporter cell line, it is one of a series of reporter bioassays using CAR-T Lentivirus and Jurkat/NFAT-luciferase reporter cell lines. The anti-BCMA CAR Jurkat/NFAT-luciferase reporter cell line is a great system to predict the mechanism of action (MOA) and therapeutic potential of the anti-BCMA CAR lentivirus before using it with patient-derived primary T cells. It is a single cell clonal stable cell line developed by transducing the Jurkat/NFAT-Luciferase reporter cells with the anti-BCMA scFV CAR lentivirus (BPS Bioscience #79701).

Below is additional information on BCMA and the 3rd generation CAR (Chimeric Antigen Receptor) design. B-cell maturation antigen (BCMA) is a cell surface receptor encoded by the TNFRSF17 gene and belonging to the TNF receptor superfamily. It is preferentially expressed in certain types of B cells. BCMA is an ideal target for immunotherapy as it is nearly uniformly overexpressed by the malignant plasma cells of most patients with Multiple Myeloma (MM) while it has very low expression in other malignant or non-malignant tissues. Chimeric Antigen Receptor (CAR) T cell therapy is a cellular therapy which redirects a patient's T cells to specifically target and destroy tumor cells. CARs are genetically engineered fusion proteins consisting of (1) an antigen recognition domain (e.g. scFv) derived from a monoclonal antibody and (2) intracellular T cell signaling and costimulatory domains. Using CAR-T cells as a treatment for cancer has been most extensively investigated in patients with B cell malignancies, and early results have been encouraging. CD28 is one of the proteins expressed on T cells that provide co-stimulatory signals required for T cell activation and survival. CD28 modulates the primary TCR/CD3ζ signal in a different fashion than the costimulatory elements OX40 or 4-1BB. CD28 enhances the expression of downstream regulators that impact T-cell proliferation, death, differentiation, and effector functions. CAR+ T cells containing the CD28

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endodomain showed strikingly enhanced and sustained T cell activation, growth, survival. Including CD28 costimulatory domains in CARs has led to enhanced anti-malignancy efficacy. 4-1BB (also known as CD137) is a surface co-stimulatory glycoprotein present on activated T lymphocytes that belongs to the tumor necrosis factor (TNF) receptor superfamily. It is expressed mainly on activated CD4+ and CD8+ T cells, and binds to a high-affinity ligand (4-1BBL/CD137L) expressed on some antigen presenting cells and activated B cells. On the basis of preclinical observation, this molecule can promote the persistence of antigen-specific and antigen-nonspecific chimeric antigen receptor T-cells to significantly increases antitumor activity. CD3ζ, also known as CD3ζ or T-cell receptor zeta, forms the TCR-CD3 complex together with T-cell receptor and the CD3γ, δ, and ε chains. CD3ζ was expressed independently from the complex. The zeta chain plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways. CD3ζ, which contains 3 ITAMs, is the most commonly used endo-domain component of CARs. It transmits an activation signal to the T cell after antigen is bound. CD3ζ may not provide a fully competent activation signal so additional co-stimulatory signaling is needed. For example, chimeric CD28 or 4-1BB can be used with CD3ζ to transmit a proliferative/survival signal, or all three can be used together.

**Application**

- Anti-BCMA CAR Jurkat/NFAT-luciferase stable reporter cell line can be used to predict the Mechanism of Action (MOA) of the CAR design and construct in CAR-T therapeutic development.
- Anti-BCMA CAR Jurkat/NFAT-luciferase stable reporter cell line can provide accurate and precise measurements of antibody (scFV-CAR) specificity and potency before going into patient-derived T cells.
- Anti-BCMA CAR Jurkat/NFAT-luciferase stable reporter cell line can be used to screen and validate BCMA-expressing caner target cells for functional activation of the NFAT pathway in the effector cells through anti-BCMA CAR.

**Host Cell**
Jurkat NFAT-luciferase reporter cells (BPS Bioscience #60621)

**Format**
Each vial contains 2 x $10^6$ cells in 1 ml of 10% DMSO and 90% FBS

**Storage**
Immediately upon receipt, store in liquid nitrogen.

**Mycoplasma Testing**
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

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**General Cell Culture Conditions:**

**Thaw Medium 2 (BPS Bioscience, #60184):** RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

**Growth Medium 2H (BPS Bioscience, #79784):** Thaw Medium 2, plus 1 µg/ml puromycin (InvivoGen # ant-pr-1) and 1 mg/ml of Geneticin (Thermo Fisher, #11811031).

Quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 **(no Geneticin or puromycin)**. Spin down the cells, remove supernatant and resuspend cells in 5 ml pre-warmed Thaw Medium 2 **(no Geneticin or puromycin)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% $CO_2$ incubator. At first passage, switch to complete growth medium **(contains Geneticin and puromycin)**. Passage the cells at 1:10 ratio twice a week when cells are more than 2 x $10^6$ cells/ml. We recommend storing at least 10 or more vials of cells at an early passage.

**Figure 1. Anti-BCMA CAR design and lentivector:**

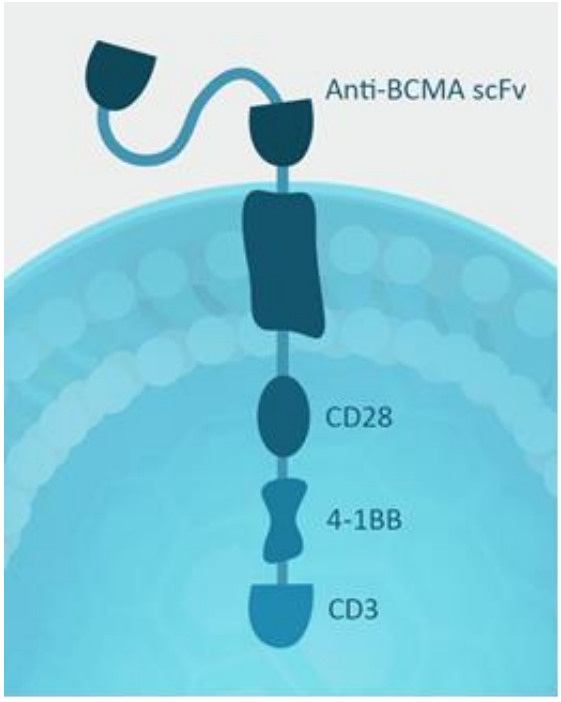

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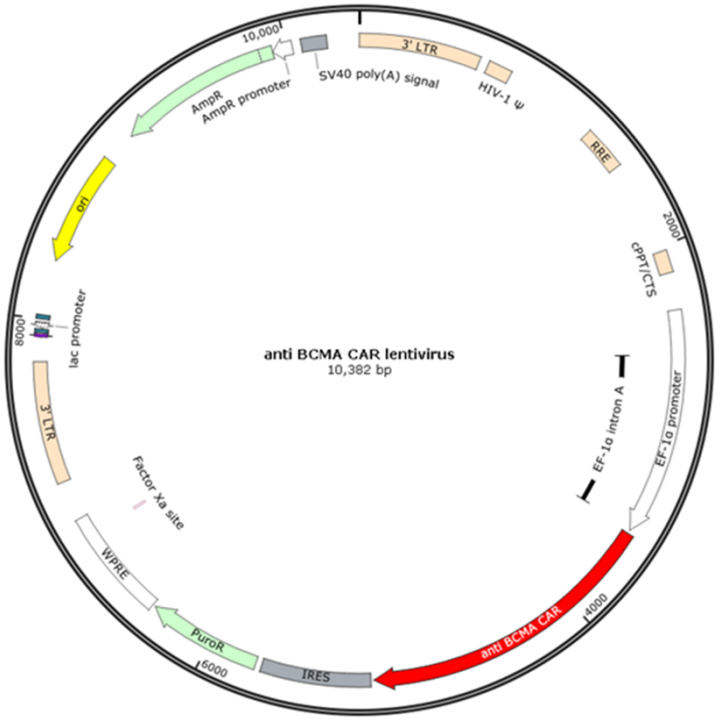
**Figure 2. Schematic of the lenti-vector used to generate the anti-BCMA CAR/ Jurkat NFAT-luciferase stable cell line**
The anti-BCMA (scFv) is linked to the 3rd generation CAR with CD28 transmembrane and costimulatory domains, 4-1BB, and CD3ζ components.




**Materials Required but Not Supplied**

- CHO-K1 cell line (ATCC) and target cell BCAM/CHO stable cell line (BPS Bioscience #79500)
- Thaw Medium 3 (BPS Bioscience #60186): Ham's F-12 medium (Hyclone # SH30526.01) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).
- Growth Medium 3D (BPS Bioscience #79539): Thaw Medium 3 plus 1 mg/ml Geneticin (Thermo Fisher, #11811031).
- Jurkat/NFAT-luciferase reporter cell line (BPS Bioscience #60621)
- Thaw Medium 2 (BPS Bioscience #60184): RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).
- Growth Medium 2H (BPS Bioscience #79784): Thaw Medium 2 plus 1 µg/ml puromycin (InvivoGen # ant-pr-1) and 1 mg/ml of Geneticin (Thermo Fisher, #11811031).
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- One-Step luciferase assay system (BPS Bioscience #60690)
- Luminometer

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**Functional Validation and Assay Performance:**

1) **Expression of anti-BCMA CAR in Jurkat NFAT-luciferase reporter cell line was confirmed by FACS.**

To measure anti-BCMA CAR expression, 0.25 million cells were suspended in 100 µl of cell staining FACS buffer with blocking antibody and incubated on ice for 10 minutes. Then 0.3 µg of biotinylated human BCMA protein (BPS Bioscience #79467) was added, and the cells were incubated on ice for 30 minutes. The cells were rinsed with 3 ml of FACS buffer, suspended in 100 µl of buffer with 5 µl of phycoerythrin (PE)-conjugated streptavidin (Biolegend), and the cells were incubated on ice for 30 minutes. The cells were rinsed with 3 ml of buffer, then suspended in 100 µl FACS buffer with 5 µl of 7-AAD (BioLegend), and analyzed on a NovoCyte flow cytometer.

**Figure 3.** FACS analysis of cell surface expression of anti-BCMA CAR in anti-BCMA CAR /NFAT Reporter-Jurkat cells using biotinylated human BCMA protein-biotin (BPS Bioscience #79467) and phycoerythrin (PE)-conjugated streptavidin (Biolegend).

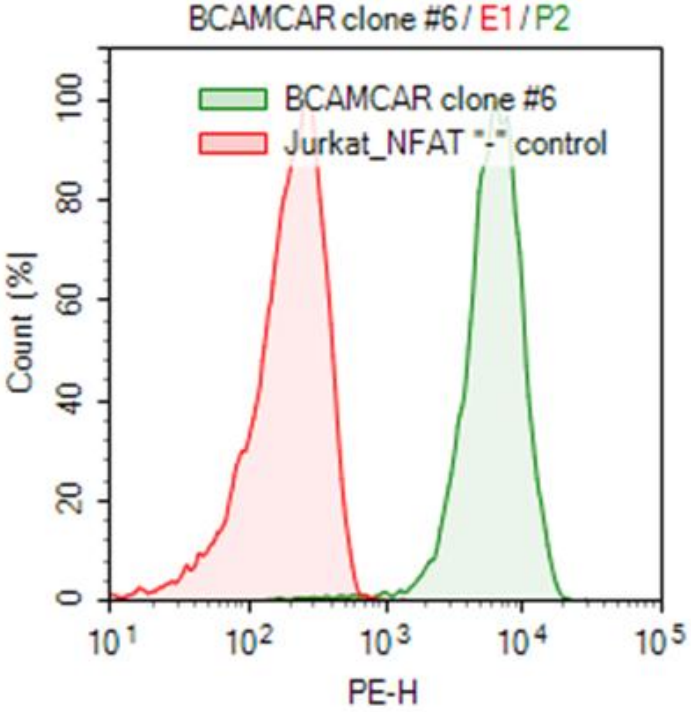

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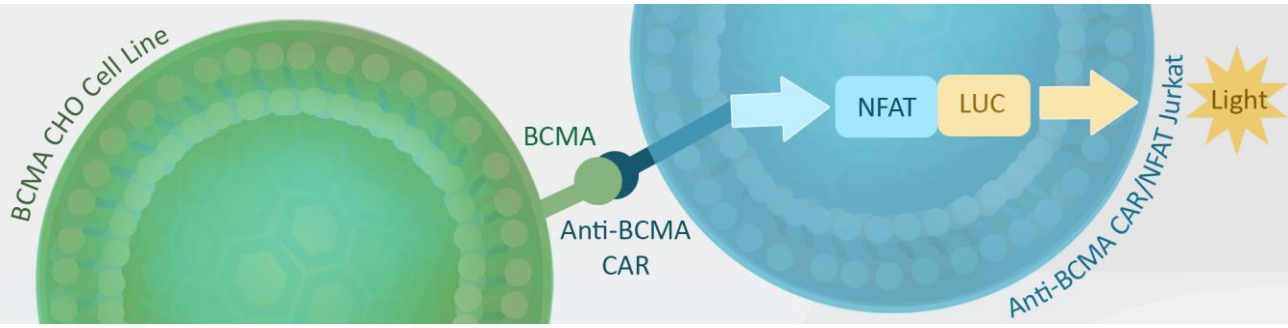
**Figure 4. Functional luciferase assay to test the specificity and potency of anti-BCMA scFv CAR/Jurkat NFAT reporter cell line using BCMA/CHO target cells co-culture:**




**Assay Protocol:**

a. Seed wild-type CHO (negative control) or BCMA/CHO target cells in triplicates at 30,000 cells per well in 100 µl Thaw Medium 3 in a 96 well white wall clear bottom plate. Incubate at 37°C for 6-12 hours to allow the cells to adhere.
b. The next day, remove Thaw Medium 3 and add anti-BCMA CAR NFAT reporter cells at 30,000 cells/well (100 µl) in Thaw Medium 2. Add soluble BCMA protein to test wells, or just Thaw Medium 2 for control wells. Incubate at 37°C with 5% $CO_2$ for 6-16 hours.
c. Lyse and cells and perform luciferase assay: prepare the ONE-Step™ Luciferase reagent (BPS Bioscience #60690) per the recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.
d. Soluble BCMA caused increase of luciferase activity by 4-fold and BCMA/CHO cells caused a 23-fold increase, by activation of NFAT through CD3ζ signaling domain downstream of anti-BCMA CAR.

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**Figure 5. Anti-BCMA CAR NFAT reporter stable cell line activity stimulated by soluble BCMA or BCMA in CHO cells**. Both soluble BCMA protein and BCMA/CHO cells caused increase of luciferase activity (4-fold with soluble BCMA and 23-fold with BCMA/CHO) by activation of NFAT through CD3ζ signaling domain downstream of anti-BCMA CAR.

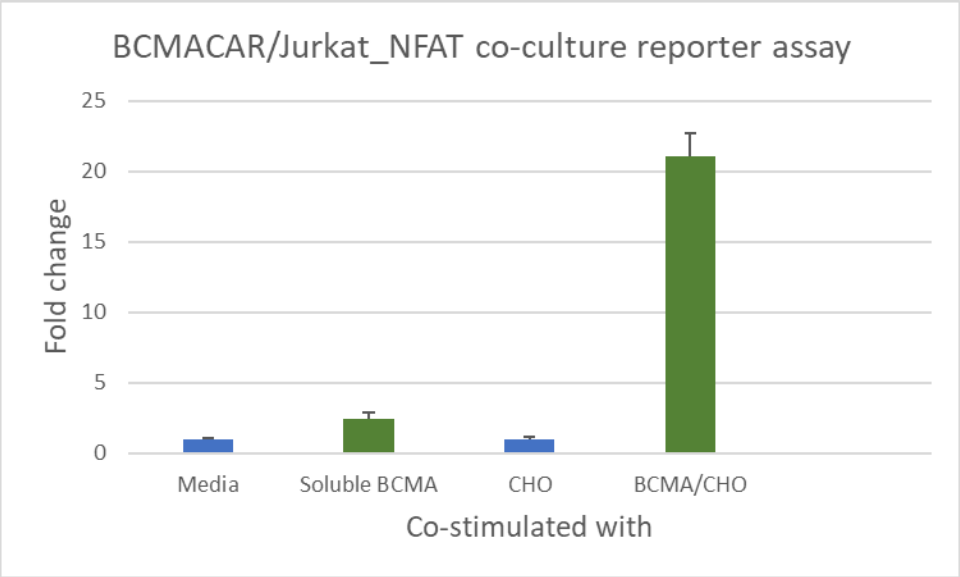
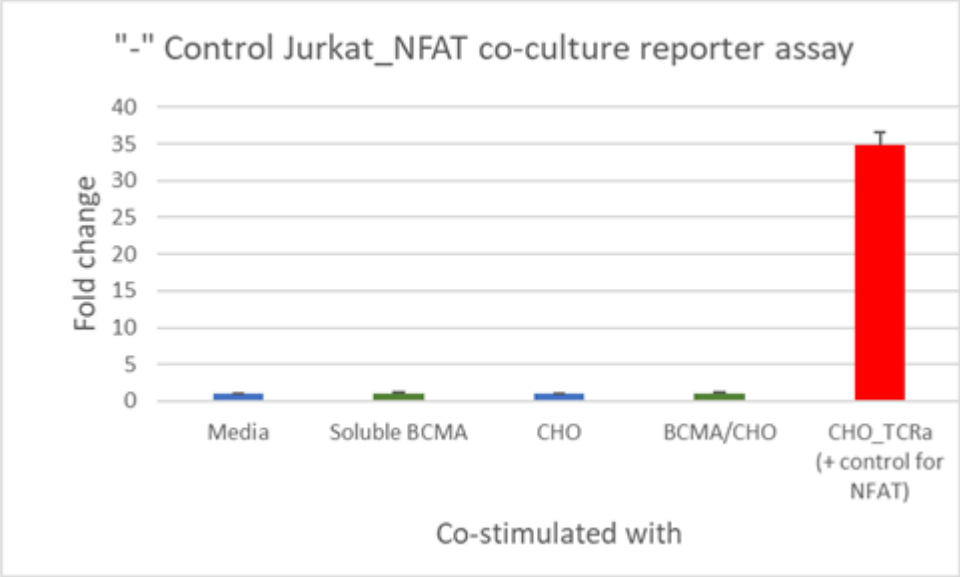



**Figure 6. The control Jurkat NFAT reporter cell line** did not respond to either soluble BCMA or BCMA in CHO cells. It only responded CHO TCRa cells expressing anti-CD3ζ domain through the endogenous TCR on Jurkat cells.



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| **Related Products** | **Cat. #** | **Size** |
|---|---|---|
| BCMA protein-biotin | 79467 | 50 µg |
| BCAM/CHO stable cell line | 79500 | 2 vials |
| anti-BCMACAR lentivirus | 79701 | 2 x 500 µl |
| Colorimetric Human IFN-γ Detection Kit | 79777 | 96 rxns. |
| ONE-Step Luciferase Detection Reagent | 60690-1 | 10 ml |
| Jurkat/NFAT-luciferase reporter cell line | 60621 | 2 vials |
| Thaw Medium 2 | 60184 | 100 ml |
| Thaw Medium 3 | 60186 | 100 ml |

**References**

1. Immune checkpoint blockade and CAR-T cell therapy in hematologic malignancies. Wang et. al. *J Hematol Oncol. 2019 Jun 11;12(1):59*
2. Chimeric antigen receptor T cell therapy for multiple myeloma. Hasegawa et.al. *Inflamm Regen. 2019 Jun 4;39:10.*
3. Novel targets for the treatment of relapsing multiple myeloma. Giuliani et. al. *Expert Rev Hematol. 2019 Jun 3:1-16*.
4. Anti-BCMA antibodies in the future management of multiple myeloma. Gavriatopoulou et. al. *Expert Rev Anticancer Ther. 2019 Apr;19(4):319-326.*