Description

Anti-CD19 CAR Negative Control/NFAT (Luciferase) Reporter Jurkat Cell Line (CD19 SCFV-CD28 Transmembrane Motif) is a stable cell line expressing an anti-CD19 CAR negative control and an NFAT-dependent luciferase reporter. The anti-CD19 CAR negative control consists of anti-CD19 scFv linked to the CD28 transmembrane motif **without** the intracellular signaling domains. The reporter cell line has been validated for anti-CD19 expression by flow cytometry, and in co-culture with target cells, such as CD19/CHO recombinant cell line the luciferase reporter gene was not activated. Anti-CD19 CAR Negative Control Jurkat/NFAT (Luciferase) Reporter Cell Line was generated by the transduction of NFAT Luciferase Reporter Jurkat Cell Line (BPS Bioscience #60621) with anti-CD19 CAR negative control lentivirus.

The cell line can be used as negative control for the Anti-CD19 CAR/ NFAT (Luciferase) Reporter Jurkat Cell Line (CD19 SCFV-CD28-4-1-BB-CD3ζ) (BPS Bioscience #79853).

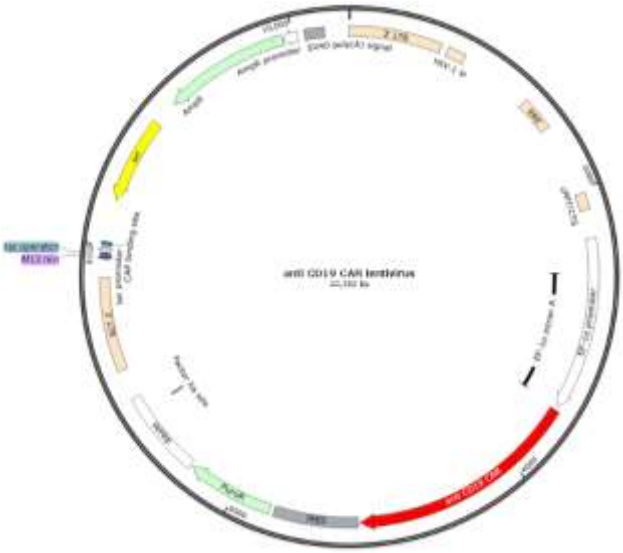

Figure 1: Lenti-vector used to generate anti-CD19 CAR negative control lentivirus.

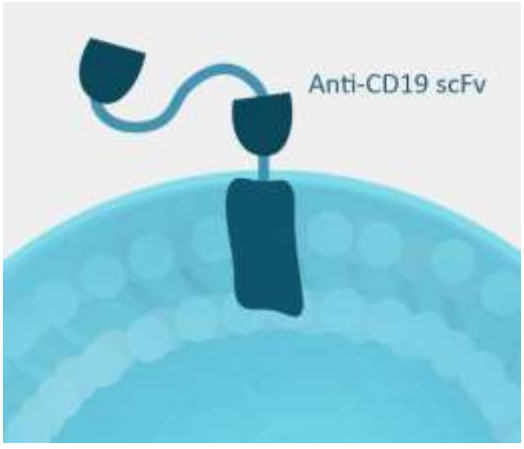

Figure 2. Schematic of anti-CD19 CAR negative control.
The anti-CD19 (scFv) is linked to the CD28 transmembrane motif.

Background

CD19 (also known as Cluster of Differentiation 19, B-lymphocyte surface antigen B4, or CVID3) is a glycoprotein expressed at the surface of B lymphocytes through most phases of B cell maturation. It is strictly required for B cell terminal differentiation. Mutations in the CD19 gene cause severe immune-deficiency syndromes associated with impaired antibody production, such as CVID3 (common variable immuno-deficiency 3). The majority of B cell malignancies express normal to high levels of CD19, making it a nearly ideal target for cancer immunotherapy. Blinatumomab, a CD19/CD3 bi-specific T cell engager (BiTE) has been approved for relapsed/refractory B precursor ALL (Acute lymphoblastic leukemia) and CD19 was the target of the first approved CAR-T cell therapy. Studies of CD19 function and expression profiles will continue to broaden our knowledge and support further applications in cancer therapy.

Application

Use as negative control for the anti-CD19 CAR/ NFAT (Luciferase) Reporter Jurkat Cell Line (CD19 SCFV-CD28-4-1-BB-CD3ζ) (BPS Bioscience #79853)

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml in Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

Jurkat (clone E6-1), human T lymphoblast, suspension

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 2	BPS Bioscience #60184
Growth Medium 2H	BPS Bioscience #79784

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Thaw Medium 3	BPS Bioscience #60186
CD19, Fc-Fusion (IgG1), Avi-Tag, Biotin labeled	BPS Bioscience #79475
CD19 CHO Recombinant Cell Line	BPS Bioscience #79561
NFAT Reporter (Luc)- Jurkat Recombinant Cell Line	BPS Bioscience #60621
Anti-CD19 CAR / NFAT (Luciferase) Reporter Jurkat Cell Line (CD19 SCFV-CD28-4-1BB-CD3ζ)	BPS Bioscience #79853
Empty vector control – CHO-K1 Recombinant Cell line	BPS Bioscience #60545
PE Streptavidin	Biolegend #405203
7-AAD	BioLegend #420403
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 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2H (BPS Bioscience #79784):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, plus 1 µg/ml Puromycin and 1 mg/ml of Geneticin.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Thaw Medium 3 (BPS Bioscience #60186):
F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: Jurkat cells are derived from human material and thus the use of adequate safety precautions is recommended.

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 2 to the conical tube containing the cells. Thaw Medium 2 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 2.

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 attachment and viability. Change medium to fresh Thaw Medium 2 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to be split.

7. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2H.

Cell Passage

Dilute the cell suspension into new cell culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.5 x 10^6 cells/ml, with Growth Medium 2H. The sub-cultivation ratio used should maintain the cells between 0.5-2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. 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.

3. 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.

Functional Validation

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.

A. Flow Cytometry analysis of anti-CD19 CAR expression.

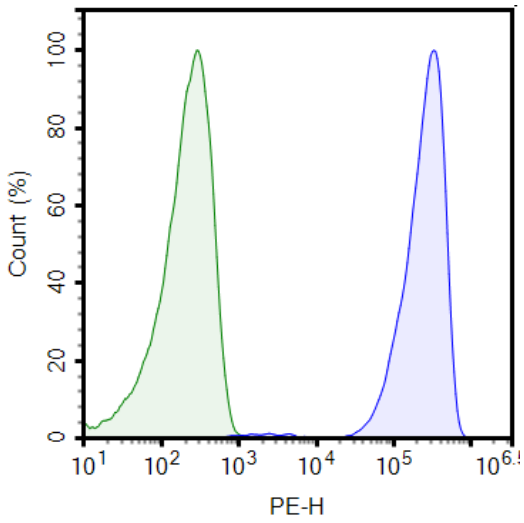

Figure 3. *Expression of anti-CD19 CAR negative control in Anti-CD19 CAR Negative Control/NFAT (Luciferase) Reporter Jurkat Cell Line.*
250,000 Anti-CD19 CAR Negative Control/ NFAT (Luciferase) Reporter Jurkat cells and parental cells were stained with 2 µg of 20 µg/ml biotinylated human CD19 protein (BPS Bioscience #79475) for 30 minutes on ice. Cells were washed and incubated with 1 µg of 10 µg/ml phycoerythrin (PE)-conjugated streptavidin (Biolegend #405203) for 30 minutes on ice. Cells were washed and resuspended in 100 µl of buffer containing 5 µl of 7-AAD (BioLegend #420403) before analysis by flow cytometry. Anti-CD19 CAR Negative Control/NFAT (Luciferase) Reporter Jurkat cells are shown in blue and NFAT (Luciferase) Reporter Jurkat cells are shown in green.

B. Co-culture assay of Anti-CD19 CAR Negative Control/NFAT (Luciferase) Reporter stable cell line, or control cell line, with CD19 expressing cells.

- This experiment measures the effect on reporter activation of co-cultivating anti-CD19 CAR Negative Control/NFAT (Luciferase) Reporter cells with CD19-expressing cells.
- All samples and controls should be performed in triplicate.

Day 1:

1. Seed Empty vector control – CHO-K1 cells (BPS Bioscience #60545) or CD19 CHO cells (BPS Bioscience #79561) at a density of 30,000 cells per well in 100 µl of Thaw Medium 3 into a white, clear-bottom 96-well culture plate. Incubate the cells at 37°C with 5% CO_2 overnight. Keep three wells without cells as "Background Luminescence Control".

Day 2:

1. Remove Thaw Medium 3 and add 50,000 cells per well in 100 µl of Thaw Medium 2 of anti-CD19 CAR /NFAT (Luciferase) Reporter cells (BPS Bioscience #79853), anti-CD19 Negative Control/NFAT (Luciferase) Reporter cells (BPS Bioscience #79854) or NFAT Reporter (Luc) – Jurkat cells (BPS Bioscience #60621).

Day 3:

1. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at RT for ~30 minutes.

2. Measure luminescence using a luminometer.

3. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the average luminescence reading of all wells. The fold induction of luciferase reporter expression is the background-subtracted luminescence of Jurkat cells cultured with CD19 CHO cells (stimulated) divided by the average background-subtracted luminescence of Jurkat cells cultured with CHO cells (unstimulated).

$$Fold\ induction = \frac{(luminescence\ stimulated\ cells - background)}{(luminescence\ unstimulated\ cells - background)}$$

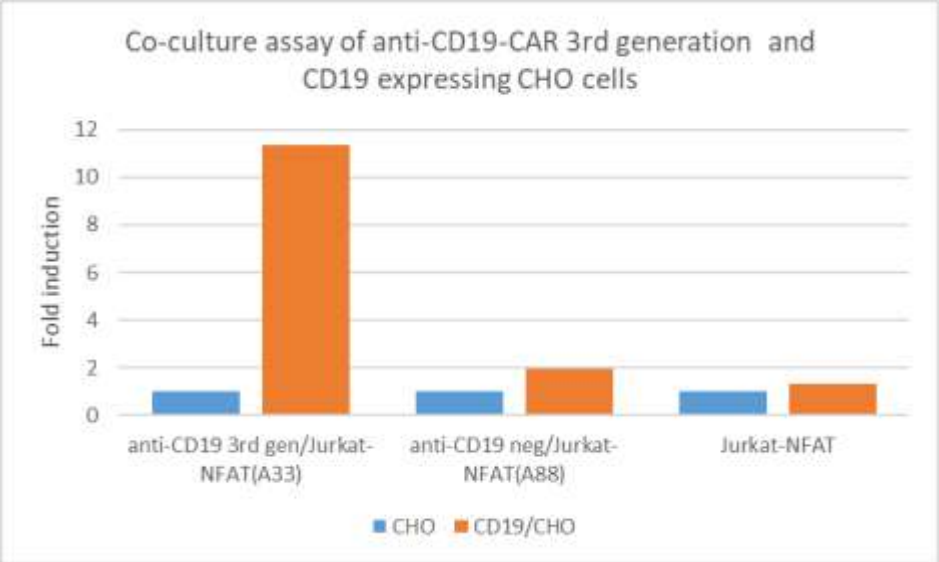

Figure 4: Activation of anti-CD19 CAR/NFAT Luciferase Reporter cell line by CD19-expressing cells. Empty vector control – CHO-K1 and CD19 CHO cells were incubated with Anti-CD19 CAR/NFAT (Luciferase) Reporter Jurkat cells, anti-CD19 CAR Negative Control/NFAT (Luciferase) Reporter Jurkat cells, and Jurkat control cells. Wild-type CHO control cells did not activate the luciferase reporter in any condition, while CD19 CHO cell line induced luciferase activity when incubated with anti-CD19 CAR/NFAT (Luciferase) Reporter Jurkat reporter cells. Incubation with Jurkat/NFAT Luciferase Reporter cell line, and anti-CD19 CAR Negative Control Jurkat/NFAT (Luciferase) Reporter cells did not result in luciferase expression.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Wang H, *et al.,* 2019 *J Hematol Oncol.* 12(1):59-78.
Hasegawa K. and Hosen N., 2019 *Inflamm Regen.* 39**:**10-14.
Giuliani N, *et al.,* 2019 *Expert Rev Hematol.* 12(7)**:**481-496.

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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*
NFAT Luciferase Reporter Lentivirus	79579	500 µl x 2
NFAT Luciferase-eGFP Reporter Lentivirus	78656	500 µl x 2
TIGIT/ NFTA Reporter – Jurkat Cell Line	60538	2 vials
LAG3/ NFTA Reporter – Jurkat Cell Line	71278	2 vials