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Datasheet

Anti-CD19 CAR negative control/ NFAT (Luciferase) Reporter Jurkat Cell Line (CD19 SCFV-CD28 transmembrane motif)

Catalog #: 79854

Product Description

Anti-CD19 CAR negative control/NFAT-luciferase reporter Jurkat cell line is a double stable cell line expressing anti-CD19 CAR negative control and NFAT-luciferase reporter. The anti-CD19 CAR negative control consists of anti-CD19 scFv linked to the CD28 transmembrane motif **without** any intracellular signaling domains. The reporter cell line has been validated for anti-CD19 expression by FACS, while the stimulation by target cells including CD19/CHO recombinant cell line has not activated the luciferase reporter gene in this cell line. The cell line can be used for the negative control of anti-CD19 CAR/Jurkat-NFAT cell line (BPS Bioscience, #79853).

Background

The development of CAR-T cells is a complex process that requires multiple steps in the workflow including I) screening and sequencing of mAbs that are specific to the cancer antigens; II) engineering and validation of scFv and scFv-CAR of different varieties for their specificities and activities; III) production of high titer lentivirus for CAR constructs; IV) isolation, activation and expansion of primary T cells from healthy donors or patients that exhibit a specific cellular phenotype; V) transduction of activated T cells with CAR-encoding lentivirus; VI) validation of engineered CAR-T cells through FACS and functional analysis.

BPS Bioscience has developed a series of CAR-T products, including lentiviruses, reporter cell lines and fully validated functional CAR-T cells for a variety of targets such as CD19 and BCMA. In this product, anti-CD19 CAR negative control and NFAT-luciferase reporter are co-transfected into a Jurkat cell line, where the anti-CD19 scFv binds to CD19, however, it does not induce the activation of CAR and luciferase reporter through NFAT as the intracellular activation motifs are missing. Anti-CD19 scFv linked to the CD28 transmembrane region was cloned into a lentivector, and packaged using a safe, replication incompetent, and VSV-G pseudotyped lentiviral packaging system, in which the gene of anti-CD19 CAR negative control is driven by an EF-1α promotor. Anti-CD19 CAR negative control Jurkat/NFAT reporter cell line was generated by the transduction of anti-CD19 CAR negative control lentivirus into an NFAT-luciferase reporter Jurkat cell line. In these cells, the luciferase reporter should not be activated upon co-culture with CD19/CHO target cells (BPS Bioscience #79561).

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Anti-CD19 CAR/NFAT-luciferase reporter Jurkat cell line (BPS Bioscience, #79853) is a great system for primary screening of anti-CD19 CAR and predicting its mechanism of action before testing on patient-derived primary T cells. The same anti-CD19 CAR lentivirus (BPS Bioscience, #79851) was also used to transduce primary T cells to make primary anti-CD19 CAR-T cells, which showed IFN-γ production and cytotoxic killing of CD19+ tumor cells in co-culture experiments, indicating that there is a good correlation between the reporter activity in CAR reporter Jurkat cell line and functional activation of primary CAR-T cells when co-cultured with target cells.

Application

- Validate different CAR designs and constructs for their specificity, efficacy and potency before proceeding into patient-derived primary T cells.
- Intracellular co-stimulatory and activation domain comparison.
- Compound and Ab screening for modulation of CAR signaling pathways.
- Screen and validate CD19-expressing target cells for antigen-specific CAR activation.

Host Cell
NFAT-luciferase reporter Jurkat 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 Lonza MycoAlert Mycoplasma Detection kit (Lonza, #LT07-318) to confirm the absence of *Mycoplasma* species.

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

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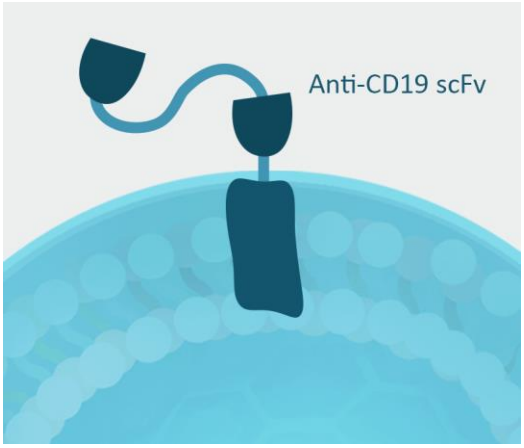
incubate at 37°C in a 5% CO_2 incubator. At first passage, switch to complete Growth Medium 2H **(contains Geneticin and puromycin)**. Passage the cells at 1:10 ratio twice a week when cells are more than 2 x 10^6 cells/ml. We recommend storing at least 10 or more vials of cells at an early passage.

Figure 1. 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.



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Materials Required but Not Supplied

- CD19, Fc Fusion, Biotin labeled (BPS Bioscience #79475)
- CD19/CHO stable cell line (BPS Bioscience #79561)
- ONE-Step™ Luciferase Detection Reagent (BPS Bioscience #60690)
- NFAT-luc reporter Jurkat cell line (BPS Bioscience #60621)
- Thaw Medium 2 (BPS Bioscience #60184)
- Thaw Medium 3 (BPS Bioscience #60186)
- Growth Medium 2H (BPS Bioscience #79784)
- 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)

Functional Validation and Assay Performance:

FACS analysis on anti-CD19 CAR expression

To determine the transduction ratio, FACS analysis with biotinylated CD19 (BPS Bioscience #79475) was performed as follows.

1) To measure anti-CD19 CAR expression, spin down 25,0000 cells and suspend in 350ul of cell staining FACS buffer to wash and spin at 300 x g for 5 minutes.
2) Add 50ul of the blocking solution and incubate for 15min at room temperature.
3) Rinse the cells with 350ul of FACS buffer
4) Stain with 2 µg of biotinylated human CD19 protein (BPS Bioscience, #79475, the final concentration is 20ug/ml) in 100 µl FACS buffer per sample and incubate on ice for 30 minutes.
5) Rinse the cells with 350ul of FACS buffer and suspend in 100 µL of FACS buffer with 1ug of phycoerythrin (PE)-conjugated streptavidin (Biolegend, #405203, final concentration is 10ug/ml).
6) After incubating on ice for 30 minutes, rinse the cells with 350ul of FACS buffer twice, then suspend in 100 µl FACS buffer with 5 µl 7-AAD (BioLegend, #420403). The cells are analyzed by NovoCyte flow cytometer.

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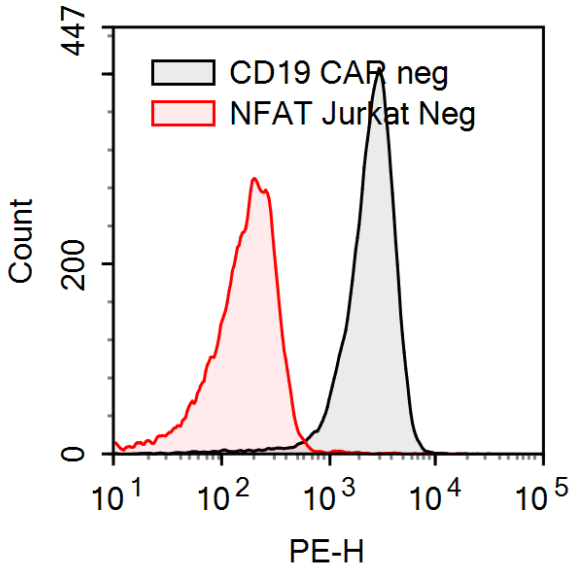
Figure 3. Expression of anti-CD19 CAR negative control in NFAT-luciferase reporter Jurkat cell line



Co-culture assay of anti-CD19 CAR NFAT reporter stable cell line activity stimulated by CD19

1) Day 1: Seed wildtype CHO cells (BPS Bioscience, #60545) or CD19-CHO cells (BPS Bioscience, #79561) at 30,000 cells per well of a white clear bottom-96 well plate in 100µl of Thaw Medium 3 (BPS Bioscience, #60186).
2) Day 2: Remove Thaw Medium 3 and add anti-CD19 CAR-Jurkat/NFAT cells (BPS Bioscience, #79853), anti-CD19 negative-Jurkat/NFAT cells (BPS Bioscience, #79854), or Jurkat/NFAT cells (BPS Bioscience, #60621) at 50,000 cells per well of a 96 well plate in 50ul of Thaw Medium 2 (BPS Bioscience, #60184).
3) Day 3: After ~16 hours, perform luciferase assay using the ONE-Step™ luciferase assay system (BPS Bioscience, #60690). Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer.

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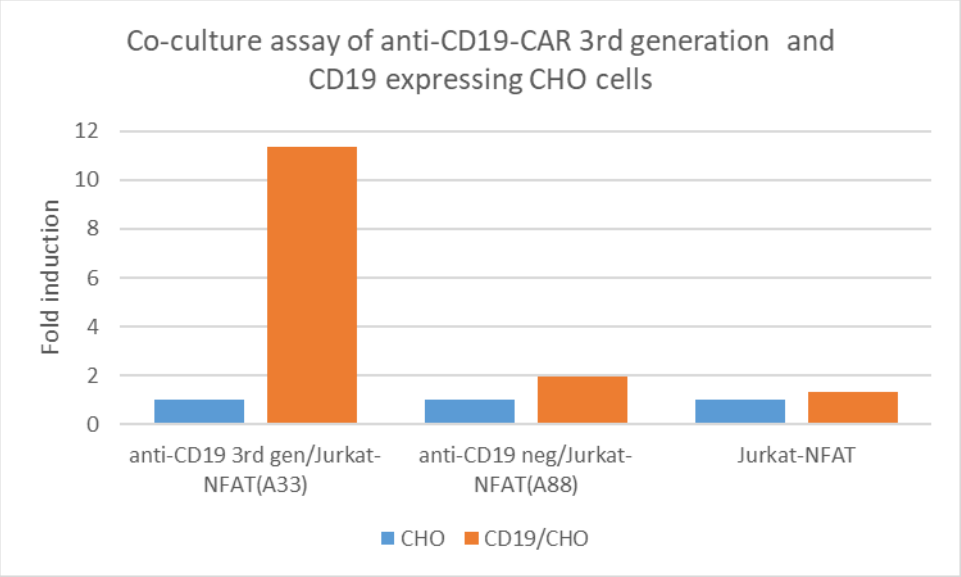
Figure 4. Co-culture assay of anti-CD19 CAR NFAT reporter stable cell line
Wildtype CHO control cells didn't show the activation, while CD19/CHO recombinant cell line induced the luciferase activity in anti-CD19 CAR NFAT Jurkat reporter cells. Jurkat/NFAT cell line and anti-CD19 CAR negative control Jurkat/NFAT cell line, which does not have any intracellular activation domain, were used as negative controls.



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Related products

anti-CD19 scFv recombinant Ab	100457	25 µg
anti-CD19 CAR lentivirus	79851	2 vials
Growth Medium 3A	60188	500 ml
CD19 / Firefly Luciferase/CHO Cell Line	79714	2 vials
Anti-CD19 CAR-CD4+ T cells	79933	1 vial
Anti-CD19 CAR-CD8 + T cells	79934	1 vial
Growth Medium 2B	79530	500 ml
Growth Medium 3D	79539	500 ml

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References

1. Immune checkpoint blockade and CAR-T cell therapy in hematologic malignancies. Wang *et al. J Hematol Oncol. 2*019 Jun 11;**12(1):**59-78.
2. Chimeric antigen receptor T cell therapy for multiple myeloma. Hasegawa *et al. Inflamm Regen.* 2019 Jun 4;**39:**10-14.
3. Novel targets for the treatment of relapsing multiple myeloma. Giuliani *et al. Expert Rev Hematol.* 2019 Jun **3:**1-16.
4. Anti-CD19 antibodies in the future management of multiple myeloma. Gavriatopoulou *et al. Expert Rev Anticancer Ther.* 2019 Apr;**19(4):**319-326.