

Datasheet

Anti-CD19 CAR / NFAT (Luciferase) Reporter Jurkat Cell Line (CD19 SCFV-CD28-4-1BB-CD3ζ)

Catalog #: AMS.79853

Product Description

Anti-CD19 CAR/NFAT-luciferase reporter Jurkat cell line is a double stable cell line expressing anti-CD19 CAR and NFAT-luciferase reporter. It is made from anti-CD19 CAR lentivirus (#AMS.79851). The reporter cell line has been validated for anti-CD19 CAR expression by FACS, and for luciferase reporter activation stimulated by target cells including CD19/CHO recombinant cell line. The reporter cell line can be used for primary screening and functional validation of anti-CD19 CAR construct and lentivirus before testing in primary T cells.

Anti-CD19 CAR consists of anti-CD19 scFv linked to 3rd generation CAR (Chimeric Antigen Receptor) containing CD28, 4-1BB co-stimulatory domains, and CD3ζ signaling domain.

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.

We have 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 and NFAT-luciferase reporter are co-transfected into a Jurkat cell line, where the interaction between CD19 and anti-CD19 scFv leads to the activation of CAR and luciferase reporter through NFAT. Anti-CD19 scFv linked to 3rd generation CAR (CD28 transmembrane and costimulatory domains, 4-1BB, and CD3ζ components) 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 is driven by an EF-1α promoter. Anti-CD19 CAR Jurkat/NFAT reporter cell line was generated by transducing anti-CD19 CAR lentivirus into an NFAT-luciferase reporter Jurkat cell line. In these cells, the luciferase reporter is activated upon co-culture with CD19/CHO target cells (#AMS.79561), or Raji cells with endogenous CD19 expression. The anti-CD19 CAR/NFAT-luciferase reporter Jurkat cell line 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

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CAR lentivirus 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 (#AMS.60621)

Format

Each vial contains 2×10^6 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 (#AMS.60184): RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 2H (#AMS.79784): Thaw Medium 2, plus 1 $\mu\text{g/ml}$ 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% CO₂ 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

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cells are more than 2×10^6 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 lentivirus

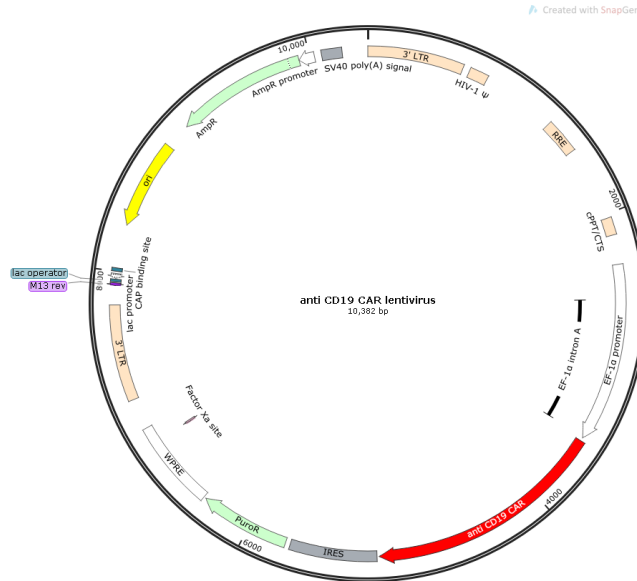
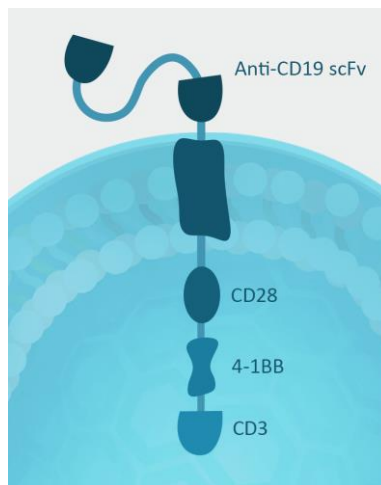


Figure 2. Schematic of anti-CD19 CAR

Anti-CD19 (scFv) is linked to the 3rd generation CAR with CD28 transmembrane and costimulatory domains, 4-1BB, and CD3 ζ components.



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Materials Required but Not Supplied

- CD19, Fc Fusion, Biotin labeled (#AMS.79475)
- CD19/CHO stable cell line (#AMS.79561)
- ONE-Step™ Luciferase Detection Reagent (#AMS.60690)
- NFAT-luc reporter Jurkat cell line (#AMS.60621)
- Thaw Medium 2 (#AMS.60184)
- Thaw Medium 3 (#AMS.60186)
- Growth Medium 2H (#AMS.79784)
- Anti-CD19 negative Jurkat/NFAT cell line (#AMS.79854)
- Empty vector control – CHO-K1 Recombinant Cell line (#AMS.60545)

Functional Validation and Assay Performance:

FACS analysis on anti CD19 CAR expression

To determine the transduction ratio, FACS analysis with biotinylated CD19 (#AMS.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 (#AMS.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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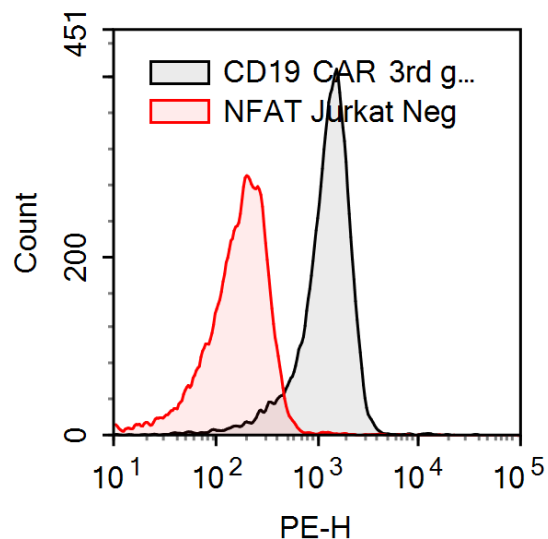
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Figure 3. Expression of anti-CD19 CAR in NFAT-luciferase reporter Jurkat cell line



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

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

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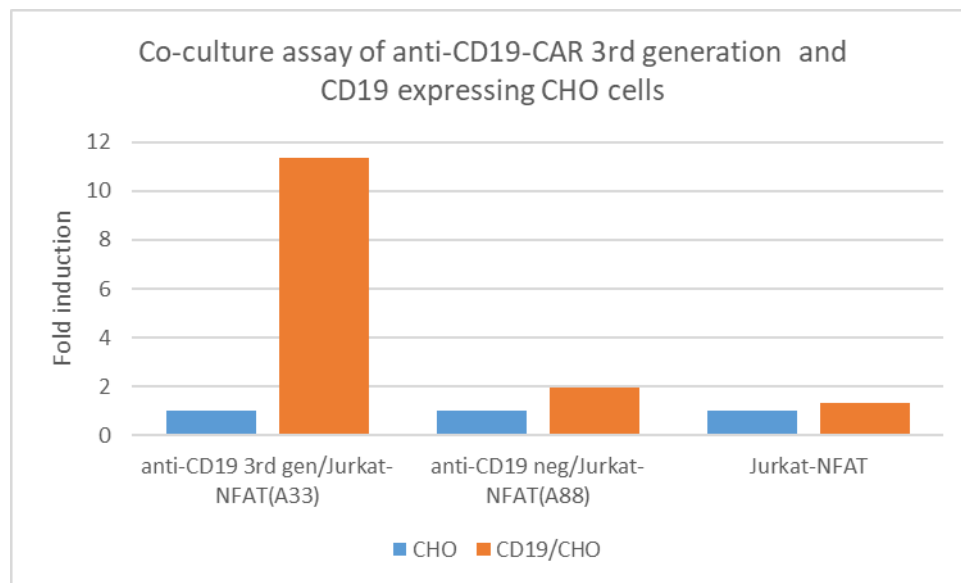
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Figure 4. Co-culture assay of anti-CD19 CAR NFAT reporter stable cell line

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.

**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

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