SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Datasheet

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

Catalog #: 79853

Product Description

The 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 the anti-CD19 CAR lentivirus (BPS Bioscience #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 and Raji cells with endogenous CD19 expression. 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.

The 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 components 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; V) 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 and NFAT-luciferase reporter are co-transfected into a Jurkat cell line, where binding of CD19 to 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, VSV-G pseudotyped lentiviral packaging system, in which the gene of anti-CD19 CAR is driven by an EF-1α promotor. The anti-CD19 CAR reporter Jurkat cell line was generated by transducing the 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 (BPS Bioscience #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

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

testing on patient-derived primary T cells. The same anti-CD19 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.
- Predict the Mechanism of Action (MOA) of CAR.
- 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.
- Proof of concept studies for primary CAR T-cells.

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 the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) 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 incubate at 37°C in a 5% CO_2 incubator. At first passage, switch to complete Growth Medium

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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 the anti-CD19 CAR lentivirus

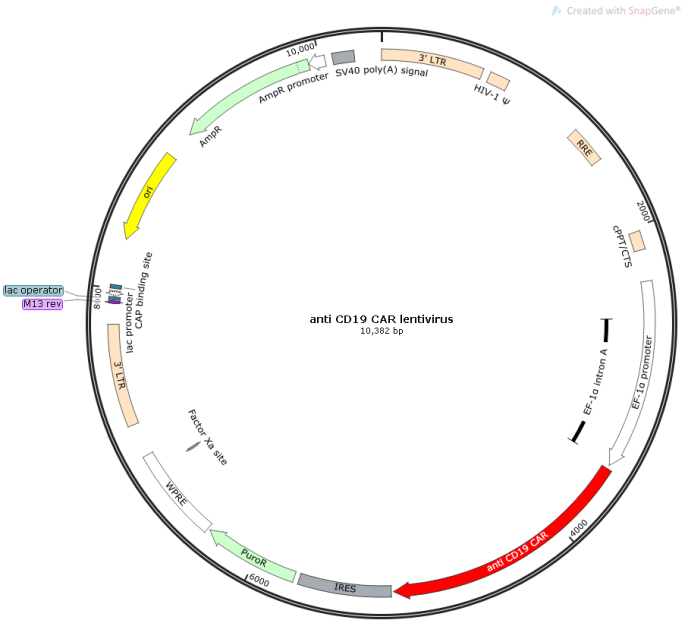
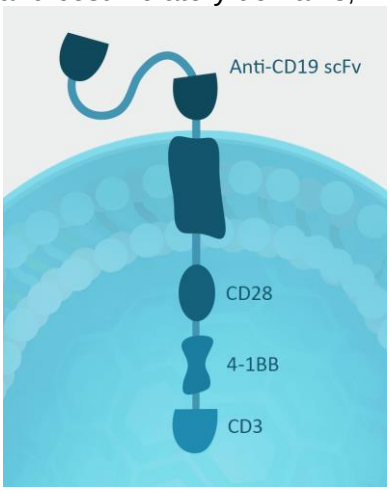


Figure 2. Schematic of anti-CD19 CAR The anti-CD19 (scFv) is linked to the 3rd generation CAR with CD28 transmembrane and costimulatory domains, 4-1BB, and CD3ζ components.



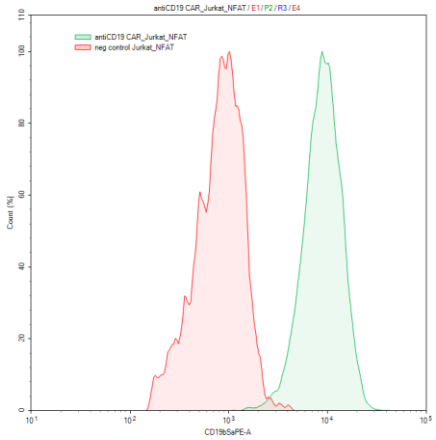
6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Materials Required but Not Supplied

- CHO-K1 cell line (ATCC), target cell CD19/CHO stable cell line (BPS Bioscience #79561), or Raji cells (ATCC, CCL-86).
- 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).
- NFAT-luciferase reporter Jurkat 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 and 1 mg/ml of Geneticin.
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- One-Step luciferase assay system (BPS Bioscience #60690)
- Luminometer

Functional Validation and Assay Performance:

Figure 3. Expression of anti-CD19 CAR in NFAT-luciferase reporter Jurkat cell line was confirmed by FACS.



Anti-CD19 CAR expression was measured using biotinylated human CD19 protein (BPS Bioscience #79467) and phycoerythrin (PE)-conjugated streptavidin (Biolegend, #405203). The cells were then analyzed on a NovoCyte flow cytometer.

.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Figure 4. Anti-CD19 CAR NFAT reporter stable cell line activity stimulated by CD19: WT CHO control cells didn't show activation, however, both CD19/CHO recombinant cell line and Raji cells with endogenous CD19 expression caused an increase of luciferase activity in the anti-CD19 CAR NFAT Jurkat reporter cells.

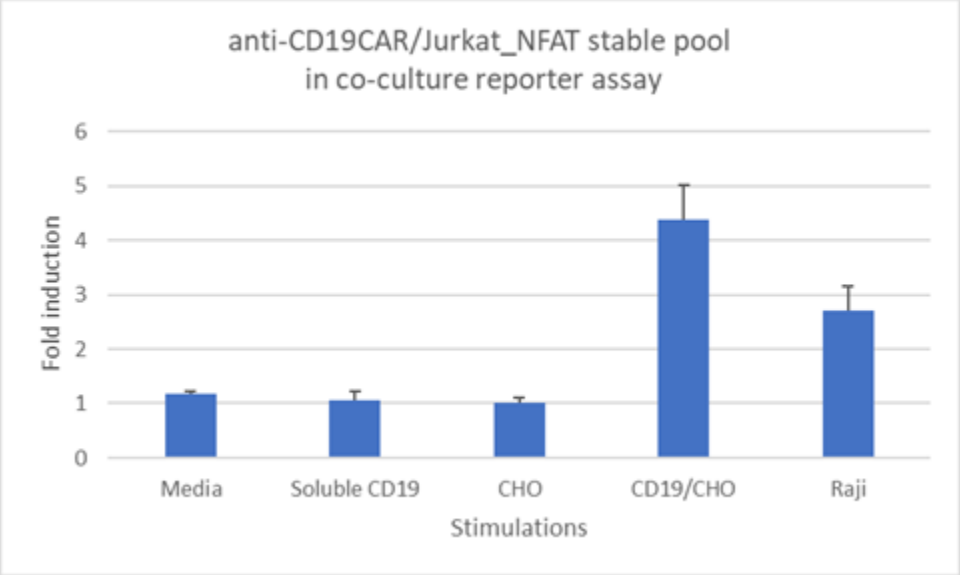
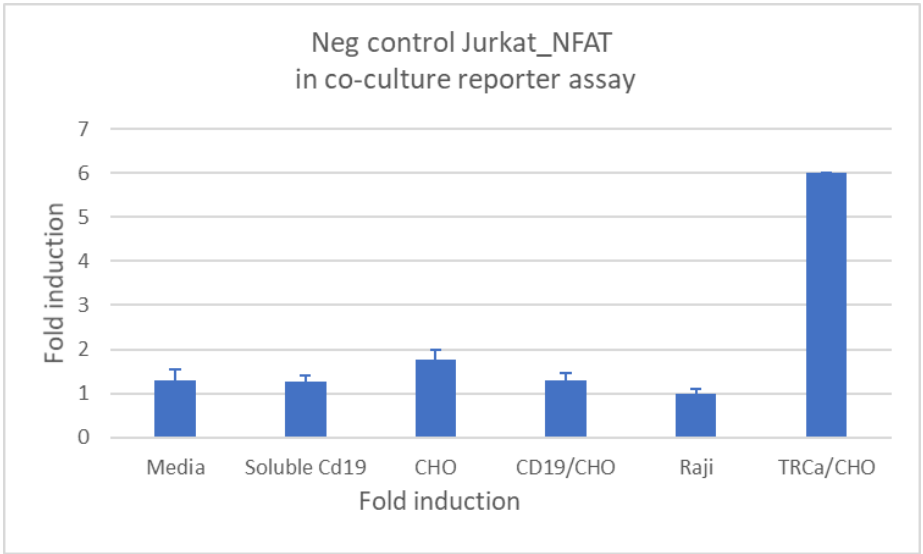


Figure 5. The negative control NFAT/Jurkat reporter cell line did not respond to CD19/CHO or Raji cells. It only responded to TCRa/CHO cells by directly activating the endogenous TCR on Jurkat cells.



6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Related Products	**Cat. #**	**Size**
CD19, Fc Fusion, Biotin labeled	79475	25 µg
CD19/CHO stable cell line	79561	2 vials
CD19/Firefly luciferase-CHO double stable cell line	79714	2 vials
anti-CD19 CAR lentivirus	79851	2 vials
Colorimetric Human IFN-γ Detection Kit	79777	96 rxns.
ONE-Step™ Luciferase Detection Reagent	60690-1	10 ml
NFAT-luc reporter Jurkat cell line	60621	2 vials
CD4+ T cells, Negatively Selected (Human)	79752	10^6 cells
CD8+ T cells, Negatively Selected (Human)	79753	10^6 cells
Thaw Medium 2	60184	100 ml
Thaw Medium 3	60186	100 ml
Thaw Medium 10	79704	100 ml
Growth Medium 2B	79530	500 ml
Growth Medium 3A	60188	500 ml
Growth Medium 3D	79539	500 ml

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.