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-CD4$^+$ T cells
(CD19 scFv-CD28-4-1BB-CD3ζ)

Catalog #: 79933
Lot#: 191112

Product Description

Anti-CD19 CAR-CD4$^+$ T cells are ready-to-use CD4$^+$ T cells infected with anti-CD19 CAR lentivirus (BPS Bioscience #79851). The anti-CD19 CAR consists of anti-CD19 scFv linked to the 3rd generation CAR (Chimeric Antigen Receptor) containing CD28, 4-1BB, and CD3ζ domains. CD4$^+$ T cells (BPS Bioscience #79752) were negatively selected from freshly isolated, primary human peripheral blood mononuclear cells (PBMCs). After CD4$^+$ T cells were infected with anti-CD19 CAR lentivirus, the IFN-γ production and cell killing assay were used to validate CAR-T activity.

Background

B-lymphocyte antigen CD19 (Cluster of Differentiation 19), also known as B-Lymphocyte Surface Antigen B4 and CVID3, is a transmembrane protein expressed in follicular dendritic cells and all B lineage cells except plasma cells. CD19 plays two major roles in human B cells. It acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane and it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways. Due to its presence on all B cells, it is a biomarker for B lymphocyte development and lymphoma diagnosis and can be used as a target for leukemia immunotherapies. CD19-targeted therapies based on T cells that express CD19-specific chimeric antigen receptors (CARs) have been utilized for their antitumor abilities in patients with CD19+ lymphoma and leukemia, such as Non-Hodgkin's Lymphoma (NHL), CLL and ALL.

CAR-T cells mediate MHC-unrestricted tumor cell killing by enabling T cells to bind target cell surface antigens through a single-chain variable fragment (scFv) recognition domain. Upon engagement, CAR-T cells form a non-classical immune synapse required for their effector function. These cells then mediate their anti-tumoral effects through perforin and granzyme, the Fas and Fas ligand, as well as the release of cytokines to sensitize the tumor stroma.

Application

- Use as a control for anti-CD19 CAR-T cells with different CD19 scFv sequences
- Use as a control for anti-CD19 CAR-T cells with different intracellular co-stimulatory and activation domains
- Proof of concept studies for primary CAR-T cells.

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Host Cell
$CD4^+$ T cells (BPS Bioscience, #79752)

Format
Each vial contains 2.5 x 10^6 cells in 1 ml of 10% DMSO and 90% FBS

Storage
Immediately upon receipt, store frozen vials of CAR-T cells in liquid nitrogen. Thawed cells should be used immediately for downstream applications. Because these are primary cells, we do not recommend maintaining these cells in culture for a long period of time.

Characterization Criteria
Cell count, viability (trypan blue exclusion and FACS with impermeable DNA binding dye), surface expression of CD3, CD4, and anti-CD19 scFv using biotinylated human CD19 (BPS Bioscience, #79475).

General Cell Culture Conditions:

Medium
BPS Thaw Medium 10 (BPS Bioscience, #79704): RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% FBS (Thermo Fisher, #26140079), 1 mM Sodium pyruvate (Corning, #25-000-CL), 1% Non-essential amino acids (Corning, #25-025-CL), 1% Penicillin/streptomycin (Thermo Fisher, #15140122)

Handling Directions
First, pre-warm BPS Thaw Medium 10 to 37°C. It is important to work quickly in the following steps to ensure high cell viability and recovery. Quickly thaw cells in a 37°C water bath with constant but gentle agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire contents to a 50 ml tube. Slowly add 10 ml of pre-warmed medium while gently swirling the tube to mix. Centrifuge the cell suspension at 300 x g for 15 minutes at room temperature. Carefully remove the supernatant with a pipette without disturbing the pellet. Gently resuspend the cell pellet by flicking the tube, then add the desired volume of warm medium, and mix. NOTE: Up to 30% cell loss can be expected during washing steps. Cells are now ready for use in downstream applications.

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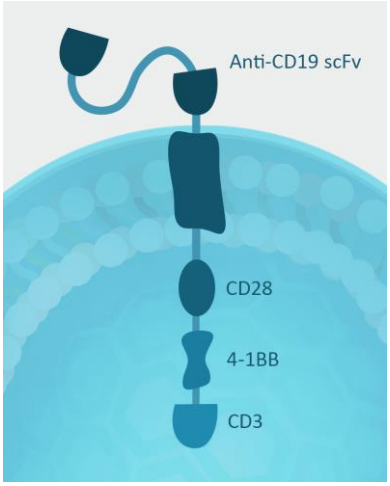
Figure 1. Schematic of anti-CD19 CAR The anti-CD19 (scFv) is linked to the 3rd generation CAR with CD28 transmembrane and costimulatory domains, 4-1BB, and CD3ζ components.



Functional Validation and Assay Performance:

FACS analysis on transduction

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 0.25 million 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 FACS.

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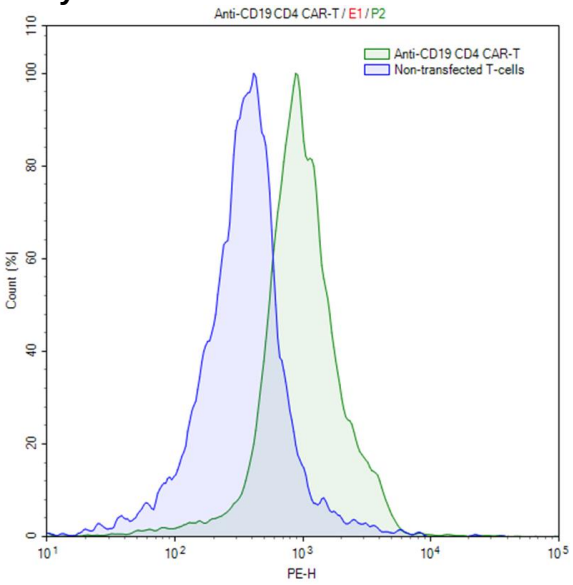
Figure2. FACS analysis on anti-CD19 CAR transduction of CD4+ T cells



IFN-γ production

To validate CAR-T activity, IFN-γ production was measured.

1) Seed target cells such as CD19/CHO cells (BPS Bioscience, #79561) and negative control cells such as regular CHO cells (BPS Bioscience, #60545) at 2,000 cells/well in a 96-well clear bottom white plate.
2) Wait until cells are attached (for CHO cells, this is usually only a few hours; for primary cells, this can be 12 hours or more) and wash away unbound cells with PBS.
3) Add CAR-T cells at an effector:target (E:T) cell ratio of 10:1.
4) After 16 hours, spin down plate, collect supernatant and detect IFN-γ expression as described in the IFN-γ detection assay using the IFN-γ (Human) Colorimetric ELISA Detection Kit (BPS Bioscience, #79777).

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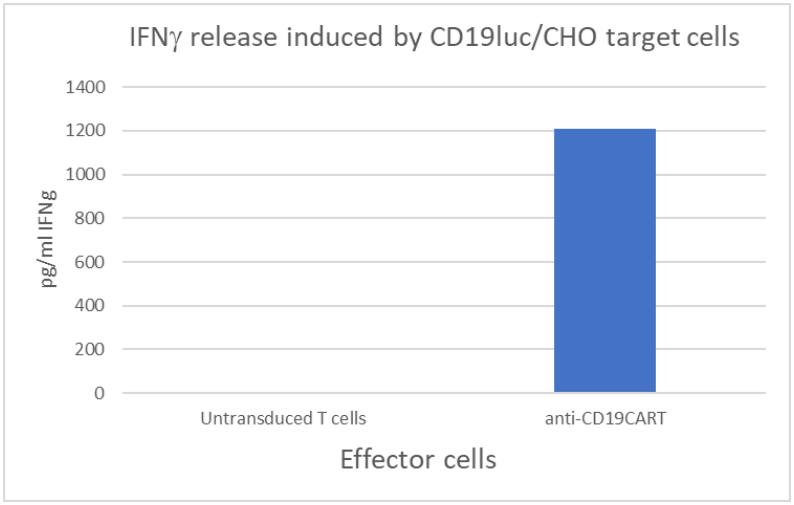
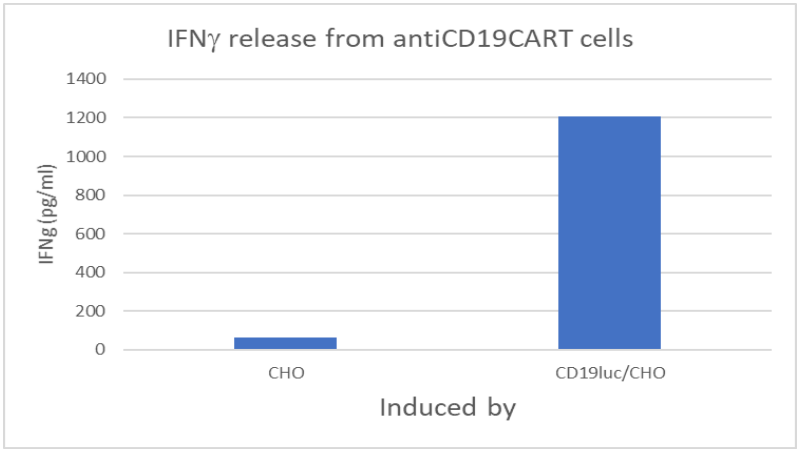
Figure 3. Comparison of IFN-γ production in non-transduced T cells and anti-CD19 CAR-T cells after the stimuli by CD19-Firefly luciferase/CHO cells



*anti-CD19 CAR-CD4$^+$ cells and -CD8$^+$ cells were mixed at 1:1 ratio for this experiment.

Figure 4. IFN-γ production of anti-CD19 CAR cells after stimuli by regular CHO cells or CD19-Firefly luciferase/CHO cells



*anti-CD19 CAR-CD4$^+$ cells and -CD8$^+$ cells were mixed at 1:1 ratio for this experiment.

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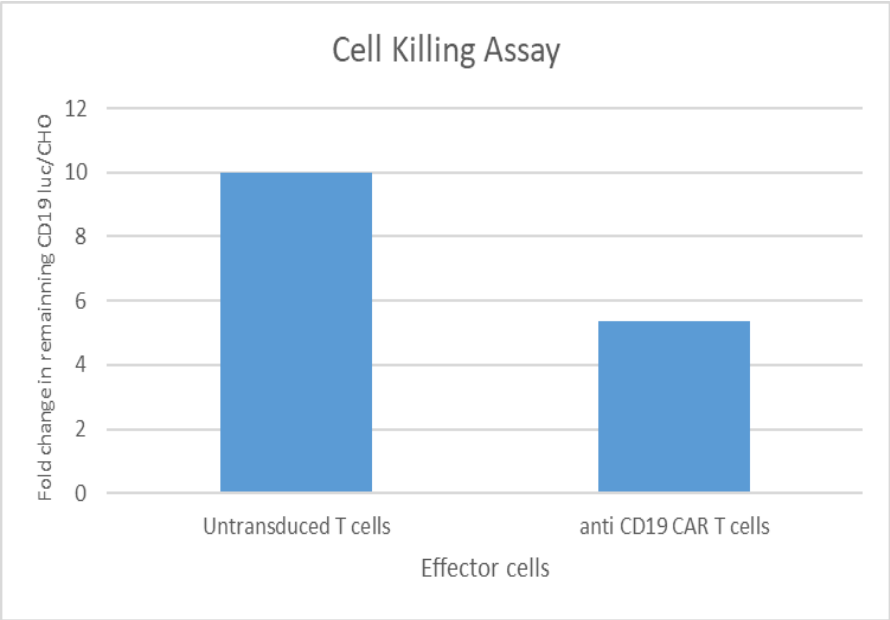
Cell killing assay

To validate CAR-T activity, cell killing was measured using luciferase activity as follows:

1) Seed target cells such as CD19-Firefly luciferase/CHO cells (BPS Bioscience, #79714) and negative control cells such as regular CHO cells (BPS Bioscience, #60545) at 2,000 cells/well in a 96-well clear bottom white plate.
2) Wait until cells are attached and wash away unbound cells with PBS.
3) Add CAR-T cells at an effector:target (E:T) cell ratio of 10:1.
4) After 16 hours, spin down plate and remove supernatant.
5) Prepare ONE-Step™ luciferase assay (BPS Bioscience, #60690) per recommended protocol. Add 100 µl of ONE-Step luciferase reagent per well and incubate at room temperature for 15 to 30 minutes.
6) Measure luminescence using a luminometer.

Figure 5. Cell Killing Assay by untransduced T cells and anti-CD19 CAR-T cells



*anti-CD19 CAR-CD4$^+$ cells and -CD8$^+$ cells were mixed at 1:1 ratio for this experiment. In this assay, less luciferase activity indicates more cell death.

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Materials required but not included	**Cat. #**	**Size**
CD19, Fc Fusion, Biotin labeled	79475	25 µg
CD19/CHO stable cell line	79561	2 vials
CD19 / Firefly Luciferase/CHO Cell Line	79714	2 vials
ONE-Step™ Luciferase Detection Reagent	60690-1	10 ml
Thaw Medium 10	79704	100ml
Empty vector control CHO-K1 cells	60545	2 vials
Colorimetric Human IFN-γ Detection Kit	79777	96 rxns.
$CD4^+$ T cells, Negatively Selected (Human)	79752	10^6 cells
Growth Medium 3B	79529	500ml
Thaw Medium 3	60186	100 ml
Growth Medium 3D	79539	500ml
Growth Medium 3A	60188	500ml
Growth Medium 2H	79784	500ml

Related products		
anti-CD19 scFv recombinant Ab	100457	25 µg
anti-CD19 CAR lentivirus	79851	2 vials
Anti-CD19 CAR-CD8 + T cells	79934	1 vial
$CD8^+$ T cells, Negatively Selected (Human)	79753	10^6 cells
Anti CD19 CAR-Jurkat/NFAT cell lines	79853	2 vials
Anti CD19 CAR negative-Jurkat/NFAT cell lines	79854	2 vials
Thaw Medium 2	60184	100 ml
Growth Medium 2H	79784	500ml

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4. Anti-CD19 antibodies in the future management of multiple myeloma. Gavriatopoulou *et al. Expert Rev Anticancer Ther.* 2019 Apr;**19(4):**319-326.

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