

Description

The AAV6 Anti-CD19 CAR (CD19 ScFv-CD8-4-1BB-CD3ζ) is an AAV 6 encoding the ScFv portion of anti-CD19 (clone FMC63) linked to the 2nd generation CAR (Chimeric Antigen Receptor) containing the CD8 hinge, 4-1BB and CD3ζ signaling domains, under the control of a EF1α promoter (Figure 1).

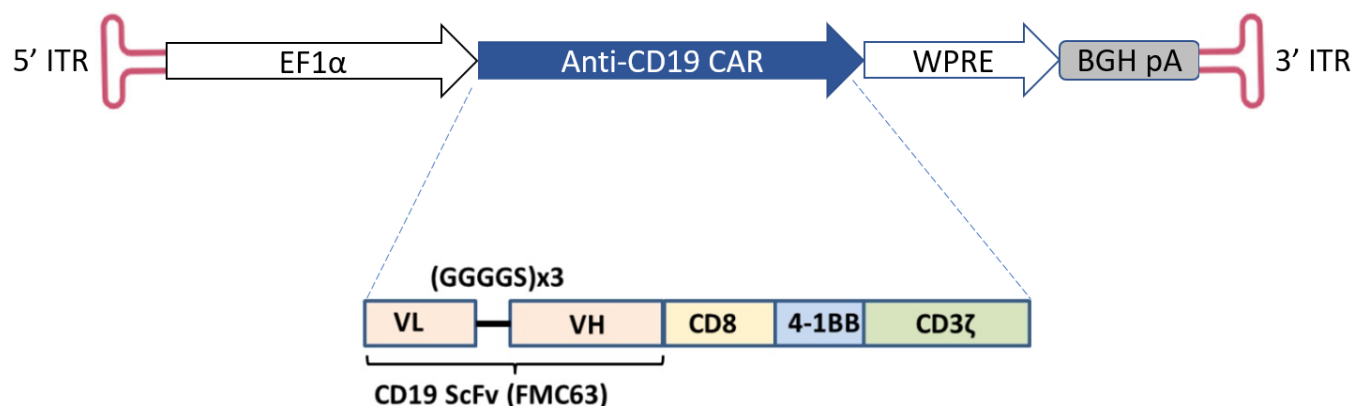


Figure 1. Schematic representation of AAV6 Anti-CD19 CAR (CD19 ScFv-CD8-4-1BB-CD3ζ). The diagram indicates the components of the anti-CD19 CAR (ScFv-CD8-4-1BB-CD3ζ).

Background

Adeno-Associated Virus Serotype 6 (AAV6) appears to be related to AAV1 by sequence analysis. In addition to skeletal muscle cells, AAV6 is more efficient at transducing airway epithelial cells in mouse lungs than AAV2 and less immunogenic, which may make AAV6 more advantageous than AAV2 in gene therapy targeting lung diseases, like cystic fibrosis. It is also able to transduce pancreatic beta-cells more efficiently than AAV1. AAV6 is able to target hematopoietic lineage cells and have been used in dendritic cells and T and B cells.

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 broader applications in cancer therapy.

Application(s)

- Positive control for anti-CD19 CAR evaluation in T cells.
- Transduction optimization experiments.
- Generate anti-CD19 CAR (chimeric antigen receptor)-T cells (for research use only, not for therapeutic purposes).

Serotype

AAV6

Formulation

AAV was produced in HEK293 cells and is supplied in PBS-MK (PBS Magnesium-Potassium) buffer containing 0.01% Pluronic F68. Viral particles can be packaged in custom formulations by special request, for an additional fee.

Purification

Purity of the AAV particles was confirmed to be greater than 90% by staining with One-Step Lumitein™ UV Protein Gel Stain (Biotium #21005-1L). The purity will vary with each lot; the exact value will be provided with each shipment.

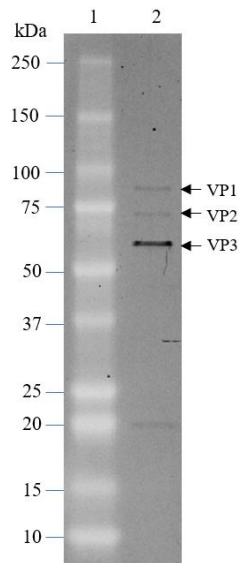


Figure 2. Purified AAV6 Anti-CD19 CAR (CD19 ScFv-CD8-4-1BB-CD3ζ) particles.

Staining of a 4-20% SDS-PAGE gel. The protein ladder was loaded on lane 1, and 2×10^9 VG (vector genome) of AAV was loaded on lane 2. AAV viral proteins VP1, VP2, and VP3 are indicated by arrows.

Size and Titer

Two vials ($50 \mu\text{l} \times 2$) of AAV at a titer $\geq 1 \times 10^{11}$ vector genomes/ml. The titer is determined by qPCR and varies with each lot; the exact value will be provided with each shipment.

Storage

AAV is shipped with dry ice. For long-term storage, it is recommended to store AAV at -80°C for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

Recombinant AAV is inherently replication-deficient and not known to cause any human diseases. Additionally, following transduction, AAV vectors exist episomally and do not integrate into or disrupt the host cell's genome. AAV requires the use of a Biosafety Level 1 facility. BPS Bioscience recommends following all local, federal, state, and institutional regulations and using all appropriate safety precautions.

Validation Data

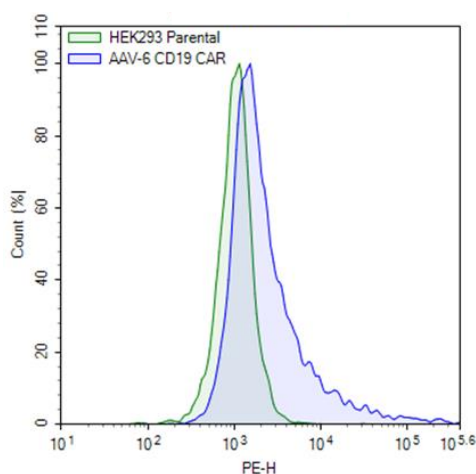


Figure 3. Expression of anti-CD19 CAR in HEK293 cells transduced with AAV6 Anti-CD19 CAR (CD19 ScFv-CD8-4-1BB-CD3ζ).

HEK293 cells were transduced with AAV6 Anti-CD19 CAR (CD19 ScFv-CD8-4-1BB-CD3ζ). Anti-CD19 expression was analyzed 5 days post-transduction by flow cytometry using PE-Labeled Monoclonal Anti-FMC63 Antibody, Mouse IgG1 (ACROBiosystems #FM3-HPY53-25tests). The HEK293 parental cells are shown in green, and HEK293 expressing anti-CD19 CAR cells are shown in blue.

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
AAV-DJ Anti-CD19 CAR (CD19 ScFv-CD8-4-1BB-CD3ζ)	82092	50 µl
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD28-4-1BB-CD3ζ, eGFP)	78775	50 µl
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD28-4-1BB-CD3ζ, SIN Vector)	78601	50 µl
Anti-CD19 CAR/ NFAT Luciferase Reporter Jurkat Cell Line (CD19 ScFv-CD28-4-1BB-CD3ζ, PuroR)	79853	2 vials
Anti-CD19 CAR Negative Control/ NFAT Luciferase Reporter Jurkat Cell Line (CD19 ScFv-CD28Transmembrane Motif)	79854	2 vials
Anti-CD19 CAR-T Cells	78171	1 vial/5 vials

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