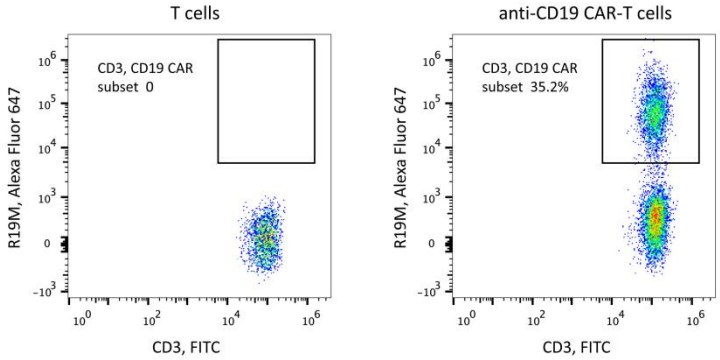
Technical Data Sheet

Rabbit Anti-Mouse FMC63 scFv Monoclonal Antibody, Alexa Fluor 647

Product Information	
Product No.	200101
Size	25 tests
Recommended Vol. per Test	1 μL
Antibody Types	Monoclonal
Antibody Format	Whole IgG
Clone	R19M
Immunogen	scFv region of a CD19-specific mouse mAb clone FMC63
Conjugate	Alexa Fluor 647
Excitation/Emission Max	651/667nm
Host Species	Rabbit
Reactivity	Mouse
Storage Buffer	Aqueous buffered solution containing protein stabilizer and ≤0.05% ProClin 300
Storage conditions	2-8°C, store in dark

Description

The rabbit monoclonal antibody R19M specifically binds to the scFv region of a CD19-specific mouse monoclonal antibody (mAb, clone FMC63). CD19 antigen is a B-cell specific cell surface antigen, which is expressed in all B-cell lineage malignancies and normal B-cells. The scFv region of FMC63 has been used to develop CD19-specific chimeric antigen receptor (CAR) T cells utilized in clinical trials.



Flow cytometric analysis of anti-CD19 CAR expression on human T cells. *Human T cells were transduced with lentivirus encoding anti-CD19 CAR and cultured for 7 days. 2×10^5 cells were stained for the expression of anti-CD19 CAR with Rabbit Anti-Mouse FMC63 scFv Monoclonal Antibody, Alexa Fluor 647 (Product No. 200101, right panel). Non-transduced T cells were used as a control for gating of CAR expression (left panel).*

Preparation & Storage

- Store undiluted at 2-8°C.
- Avoid prolonged exposure to light.
- Avoid freeze/thaw cycle of the reagent.
- The monoclonal antibody was purified by Protein A.
- The antibody was conjugated with Alexa Fluor 647 under optimum conditions, and unincorporated dye was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Antibodies to Include in the Detection Process

Product name	Product No.
Anti-human CD45 Antibody	602139/602140
Anti-human CD14 Antibody	602241
Anti-human CD8 Antibody	602006
Anti-human CD3 Antibody	603938/604045
Anti-human CD4 Antibody	601940/604240
Rabbit IgG Isotype Control, Alexa Fluor 647	700302

FACS Protocol

- If you want to set a control, the recommended reagent to use is the following: Rabbit IgG Isotype Control, Alexa Fluor 647 (Product No. 700302).

(Optional) For Whole Blood Sample

1. Pipette 1 µL Rabbit Anti-Mouse FMC63 scFv Monoclonal Antibody, Alexa Fluor 647 into the bottom of the tube.
2. Add dead cell staining solution and additional fluorochrome conjugated antibodies into the bottom of the tube.
3. Pipette 100 µL of well-mixed, anticoagulated whole blood into the bottom of the tube. Mix gently and thoroughly.

   Note Avoid smearing sample down the side of the tube. If the sample remains on the side of the tube, it will not be stained with the reagents.
4. Incubate for 25 minutes in the dark at room temperature (18-25°C).
5. Pipette Red Blood Cell Lysis Solution to the tube. Mix gently and thoroughly. Incubate for 15 minutes in the dark at room temperature (18-25°C).
6. Add 500 µL FACS buffer to the tube. Mix well and centrifuge at 300g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
7. Repeat step 6 twice.
8. Add a suitable amount of FACS buffer to resuspend cell and analysis by flow cytometry.

(Optional) For Cell Sample

1. Harvest the cells and wash the cells twice by FACS buffer.
2. Count the cells number and the viability.
3. Resuspend the cell suspension to a concentration up to 1×10^6 nucleated cells per 100 µL of buffer.
4. Add 1 µL Rabbit Anti-Mouse FMC63 scFv Monoclonal Antibody, Alexa Fluor 647, dead cell staining solution and additional fluorochrome. Mix gently and thoroughly.
5. Incubate for 25 minutes in the dark at room temperature (18-25°C).
6. Add 500 µL FACS buffer to the tube. Mix well and centrifuge at 300 g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
7. Repeat step 6 twice.
8. Add a suitable amount of FACS buffer to resuspend cell and analysis by flow cytometry.

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Caution: Antibody solutions containing ProClin 300 should be handled with care. Do not take internally and avoid all contact with the skin, mucosa and eyes.

Intellectual Product Notices

1. This product is provided under an intellectual property license from Life Technologies Corporation. The transfer of this product is conditioned on the buyer using the purchased product solely in research conducted by the buyer and the buyer must not (1) use this product or its components for (a) diagnostic, therapeutic or prophylactic purposes; or (b) manufacturing or quality assurance or quality control, and/or (2) sell or transfer this product or its components for resale, whether or not resold for use in research. For information on purchasing a license to this product for purposes other than as described above, contact Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or outlicensing@thermofisher.com.
2. Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BioSwan will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioSwan Company is strictly prohibited.
   For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resales.
   BioSwan, the BioSwan Logo and all other trademarks are property of BioSwan Laboratories, Co., Ltd.

Application References

1. Wei Wu, et al., “Multiple Signaling Roles of CD3ε and Its Application in CAR-T Cell Therapy,” Cell, 2020. 182 (4): 855-871.e23.
2. Ming Sun, et al., “Novel Two-Chain Structure Utilizing KIRS2/DAP12 Domain Improves the Safety and Efficacy of CAR-T Cells in Adults with r/r B-ALL,” Molecular Therapy – Oncolytics, 2021. 23: 96-106.
3. Sangya Agarwal, et al., “Production of Human CRISPR-Engineered CAR-T Cells,” Journal of Visualized Experiments, 2021. 169: 62299.
4. Kai Rejeski, et al., “Oligoclonal T-Cell Expansion in a Patient with Bone Marrow Failure after CD19 CAR-T Therapy for Richter-Transformed DLBCL,”

Blood, 2022. 140 (20): 2175-79.
5. Wenbin Qian, et al., “Safety and Feasibility of Anti-CD19 CAR T Cells Expressing Inducible IL-7 and CCL19 in Patients with Relapsed or Refractory Large B-Cell Lymphoma,” Blood, 2022. 140 (Supplement 1): 12722
6. Gongqiang Wu, et al., “Preclinical Evaluation of CD70-Specific CAR T Cells Targeting Acute Myeloid Leukemia,” Frontiers in Immunology, 2023. 14: 1093750.
7. Jie Cheng, et al., “Cancer-Cell-Derived Fumarate Suppresses the Anti-Tumor Capacity of CD8+ T Cells in the Tumor Microenvironment,” Cell Metabolism, 2023. 35 (6): 961-978.e10.
8. Jiali Cheng, et al., “Monitoring Anti-CD19 Chimeric Antigen Receptor T Cell Population by Flow Cytometry and Its Consistency with Digital Droplet Polymerase Chain Reaction,” Cytometry Part A, 2023. 103 (1): 16-26.
9. Kathryn R Michels, et al., “Preclinical Proof of Concept for VivoVec, a Lentiviral-Based Platform for in Vivo CAR T-Cell Engineering,” Journal for ImmunoTherapy of Cancer, 2023. 11 (3): e006292.
10. Agarwal Sangya, et al., “Deletion of the inhibitory co-receptor CTLA4 enhances and invigorates chimeric antigen receptor T cells,” Immunity, 2023. 56 (10): 2388-2407.e9.
11. Zhou H, et al. “CAR-Aptamers Enable Traceless Enrichment and Monitoring of CAR-Positive Cells and Overcome Tumor Immune Escape,” Adv Sci (Weinh). Published online, 2023 (12): e2305566.