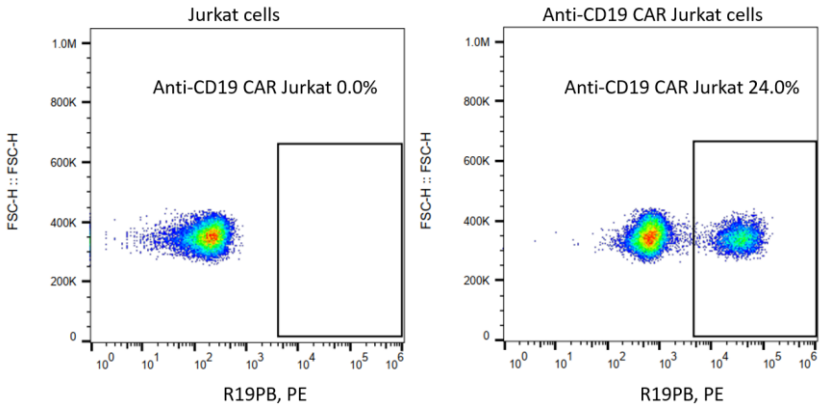
Technical Data Sheet

Rabbit Anti-Mouse FMC63 scFv Polyclonal Antibody, Biotin

Product Information	
Product No.	500014
RRID	AB_2857948
Size	100 Tests
Recommended Vol. per Test	1 μL
Antibody Types	Polyclonal
Antibody Format	Whole IgG
Immunogen	scFv region of a CD19-specific mouse mAb clone FMC63
Host Species	Rabbit
Reactivity	Mouse
Storage Buffer	Aqueous buffered solution containing protein stabilizer and ≤0.05% ProClin 300
Storage conditions	-20°C

Description

The rabbit polyclonal antibody R19P 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 cell line Jurkat cells. *Jurkat cells were transduced with lentivirus encoding anti-CD19 CAR and cultured. 2×10^5 cells were stained for the expression of anti-CD19 CAR with Rabbit Anti-Mouse FMC63 scFv Polyclonal Antibody, Biotin (Product No. 500014, right panel). Secondary staining was carried out with Streptavidin PE (Product No.700032). Non-transduced Jurkat cells were used as a control for gating of CAR expression (left panel). Acquisition of >10, 000 events was performed.*

Preparation & Storage

- Store undiluted at-20°C.
- Avoid freeze/thaw cycle of the reagent.
- The polyclonal antibody was purified by Protein A.
- The antibody was conjugated with biotin under optimum conditions, and unreacted biotin 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	602148
Anti-human CD14 Antibody	602240
Anti-human CD8 Antibody	602044
Anti-human CD3 Antibody	603943/604043
Anti-human CD4 Antibody	604344
Streptavidin PE	700032

FACS Protocol

- BioSwan reagents can be used with or without an isotype control to assess the amount of nonspecific antibody binding.

(Optional) For Whole Blood Sample

1. Pipette 1 µL Rabbit Anti-Mouse FMC63 scFv Polyclonal Antibody, Biotin into the bottom of the tube.
2. 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.
3. Incubate for 25 minutes at room temperature (18-25°C).
4. Add Streptavidin PE (Product No. 700032), dead cell staining solution and additional fluorochrome conjugated antibodies into the sample. Mix gently and thoroughly.
5. Incubate for 25 minutes in the dark at room temperature (18-25°C).
6. 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).
7. 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.
8. Repeat step 7 twice.
9. 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 Polyclonal Antibody, Biotin. Mix gently and thoroughly.
5. Incubate for 25 minutes 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. Then add 100 µL FACS buffer and mix well.
8. Add Streptavidin PE (Product No. 700032), dead cell staining solution and additional fluorochrome conjugated antibodies into the sample. Mix gently and thoroughly.
9. Incubate for 25 minutes in the dark at room temperature (18-25°C).
10. 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.
11. Repeat step 10 twice.
12. 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. 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. Jin-Yuan Ho et al., "Promoter Usage Regulating the Surface Density of CAR Molecules May Modulate the Kinetics of CAR-T Cells in Vivo," Molecular Therapy - Methods & Clinical Development 21 (June 2021): 237–46, https://doi.org/10.1016/j.omtm.2021.03.007.
2. Linfeng Yang et al., "Engineering Genetic Devices for in Vivo Control of Therapeutic T Cell Activity Triggered by the Dietary Molecule Resveratrol," Proceedings of the National Academy of Sciences 118, no. 34 (August 24, 2021): e2106612118, https://doi.org/10.1073/pnas.2106612118.

3. Ying Wang et al., “Combined 4-1BB and ICOS Co-Stimulation Improves Anti-Tumor Efficacy and Persistence of Dual Anti-CD19/CD20 Chimeric Antigen Receptor T Cells,” Cytotherapy 23, no. 8 (August 2021): 715–23, https://doi.org/10.1016/j.jcyt.2021.02.117.
4. Haiying Wang et al., “A Simple and Effective Method to Purify and Activate T Cells for Successful Generation of Chimeric Antigen Receptor T (CAR-T) Cells from Patients with High Monocyte Count,” Journal of Translational Medicine 20, no. 1 (December 19, 2022): 608, https://doi.org/10.1186/s12967-022-03833-6.
5. Jing An et al., “Enhancement of the Viability of T Cells Electroporated with DNA via Osmotic Dampening of the DNA-Sensing cGAS–STING Pathway,” Nature Biomedical Engineering, (July 27, 2023), https://doi.org/10.1038/s41551-023-01073-7.
6. Fang Xu et al., “Engineering of Dendritic Cell Bispecific Extracellular Vesicles for Tumor-Targeting Immunotherapy,” Cell Reports 42, no. 10 (October 2023): 113138, https://doi.org/10.1016/j.celrep.2023.113138.