

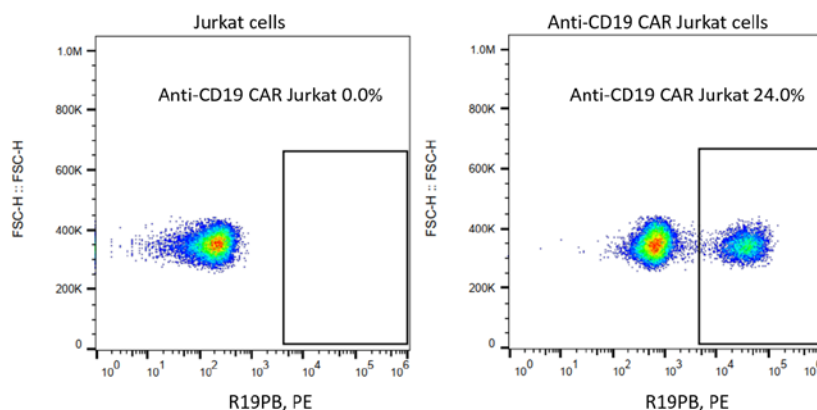
## Technical Data Sheet

### Rabbit Anti-Mouse FMC63 scFv Polyclonal Antibody, Biotin

Product Information	
Product No.	500014
RRID	AB_2857948
Size	100 tests
Recommended Vol. per Test	1 $\mu$ L
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 $\leq 0.05\%$ ProClin 300

#### 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.  $2 \times 10^5$  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 at  $-20^{\circ}\text{C}$  in small aliquot for long term storage. Avoid freeze/thaw cycle of the reagent.
- Shipped at  $2-8^{\circ}\text{C}$ . Store at  $2-8^{\circ}\text{C}$  for short term (1 month).
- 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

#### (Optional) For Whole Blood Sample

- Pipette 1  $\mu$ L Rabbit Anti-Mouse FMC63 scFv Polyclonal Antibody, Biotin into the bottom of the tube.
- Pipette 100  $\mu$ L 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.
- Incubate for 25 minutes at room temperature (18-25°C).
- Add Streptavidin PE (Product No. 700032), dead cell staining solution and additional fluorochrome conjugated antibodies into the sample. Mix gently and thoroughly.
- Incubate for 25 minutes in the dark at room temperature (18-25°C).
- 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).
- Add 500  $\mu$ L FACS buffer to the tube. Mix well and centrifuge at 300g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
- Repeat step 7 twice.
- Add a suitable amount of FACS buffer to resuspend cell and analysis by flow cytometry.

#### (Optional) For Cell Sample

- Harvest the cells and wash the cells twice by FACS buffer.
- Count the cells number and the viability.
- Resuspend the cell suspension to a concentration up to  $1 \times 10^6$  nucleated cells per 100  $\mu$ L of buffer.
- Add 1  $\mu$ L Rabbit Anti-Mouse FMC63 scFv Polyclonal Antibody, Biotin. Mix gently and thoroughly.
- Incubate for 25 minutes at room temperature (18-25°C).
- Add 500  $\mu$ L FACS buffer to the tube. Mix well and centrifuge at 300 g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
- Repeat step 6 twice. Then add 100  $\mu$ L FACS buffer and mix well.
- Add Streptavidin PE (Product No. 700032), dead cell staining solution and additional fluorochrome conjugated antibodies into the sample. Mix gently and thoroughly.
- Incubate for 25 minutes in the dark at room temperature (18-25°C).
- Add 500  $\mu$ L FACS buffer to the tube. Mix well and centrifuge at 300 g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
- Repeat step 10 twice.
- Add a suitable amount of FACS buffer to resuspend cell and analysis by flow cytometry.

### Product Notices

- Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 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

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### Application References

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- 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>.
- 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>.

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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>.