

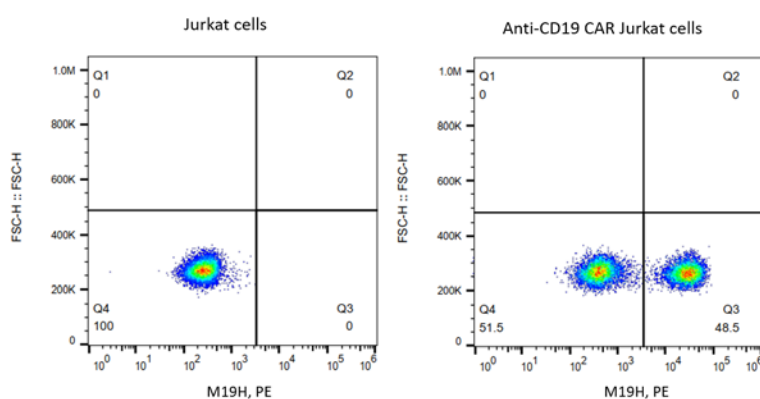
## Technical Data Sheet

### Mouse Anti-Mouse FMC63 scFv Monoclonal Antibody, Biotin

Product Information	
Product No.	300414
Size	100 tests
Recommended Vol. per Test	1 $\mu$ L
Antibody Types	Monoclonal
Antibody Format	Whole IgG
Clone	M19H
Immunogen	scFv region of a CD19-specific mouse mAb clone FMC63
Host Species	Mouse
Reactivity	Mouse
Storage Buffer	Aqueous buffered solution containing protein stabilizer and $\leq 0.05\%$ ProClin 300
Storage conditions	-20°C

### Description

The mouse monoclonal antibody M19H 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 Mouse Anti-Mouse FMC63 scFv Monoclonal Antibody, Biotin (Product No. 300414, right panel). Non-transduced Jurkat cells were used as a control for gating of CAR expression (left panel).*

### Preparation & Storage

- Store at -20°C in small aliquot for long term storage. Avoid freeze/thaw cycle of the reagent.
- Shipped at 2-8°C. Store at 2-8°C for short term (1 month).
- The monoclonal antibody was purified by Protein A.
- The antibody was conjugated with biotin under optimum conditions.

### 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 Mouse Anti-Mouse FMC63 scFv Monoclonal 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 Mouse Anti-Mouse FMC63 scFv Monoclonal 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

- Maoxuan Liu et al., "CAR-Macrophages and CAR-T Cells Synergistically Kill Tumor Cells In Vitro," Cells 11, no. 22 (November 21, 2022): 3692, <https://doi.org/10.3390/cells11223692>.