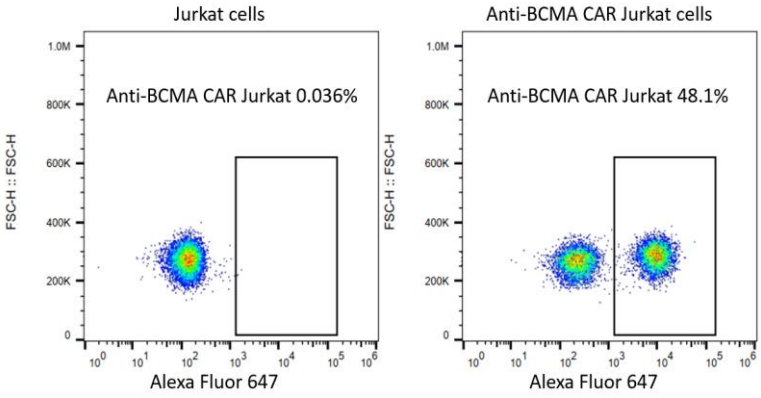
Technical Data Sheet

Rabbit Anti-Mouse C11D5.3 scFv Polyclonal Antibody, Alexa Fluor 647

Product Information	
Product No.	500502
Size	100 Tests
Recommended Vol. per Test	1 μL
Antibody Types	Polyclonal
Antibody Format	Whole IgG
Immunogen	scFv region of a BCMA-specific mouse mAb clone C11D5.3
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 polyclonal antibody specifically binds to the scFv region of a B-cell maturation antigen (BCMA) specific mouse monoclonal antibody (mAb, clone C11D5.3). BCMA is a protein that has been reported to be selectively expressed by B-lineage cells including multiple myeloma cells[1] and restrictively expressed in both normal and malignant plasma cells at high levels[2]. The scFv region of C11D5.3 has been used to develop BCMA-specific chimeric antigen receptor (CAR) T cells utilized in clinical trials.



Flow cytometric analysis of anti-BCMA CAR expression on Jurkat cells. *Jurkat cells were transduced with lentivirus encoding anti-BCMA CAR and cultured. 2×10^5 cells were stained for the expression of anti-BCMA CAR with Rabbit Anti-Mouse C11D5.3 scFv Polyclonal Antibody, Alexa Fluor 647 (Product No. 500502, right panel). 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 2-8°C.
- Avoid prolonged exposure to light.
- Avoid freeze/thaw cycle of the reagent.
- The polyclonal 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

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 C11D5.3 scFv Polyclonal 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 C11D5.3 scFv Polyclonal 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.

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References

1. Robert O. Carpenter et al., “B-Cell Maturation Antigen Is a Promising Target for Adoptive T-Cell Therapy of Multiple Myeloma,” Clinical Cancer Research 19, no. 8 (April 15, 2013): 2048–60, https://doi.org/10.1158/1078-0432.CCR-12-2422.
2. Bo Yu, et al., “BCMA-Targeted Immunotherapy for Multiple Myeloma,” Journal of Hematology & Oncology 13, no. 1 (December 2020): 125, https://doi.org/10.1186/s13045-020-00962-7.