

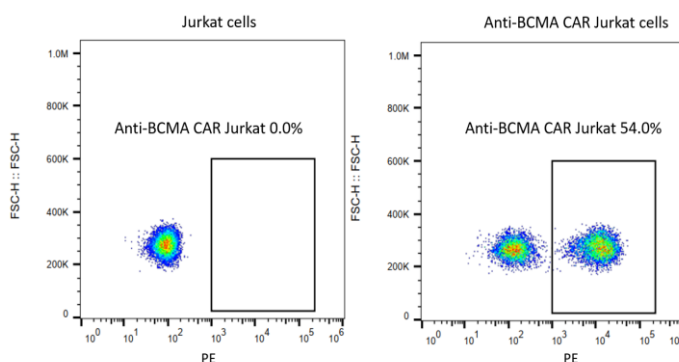
## Technical Data Sheet

### Rabbit Anti-Mouse C11D5.3 scFv Polyclonal Antibody, Biotin

Product Information	
Product No.	500514
Size	100 Tests
Recommended Vol. per Test	1 $\mu$ L
Antibody Types	Polyclonal
Antibody Format	Whole IgG
Immunogen	scFv region of a BCMA-specific mouse mAb clone C11D5.3
Host Species	Rabbit
Reactivity	Mouse
Storage Buffer	Aqueous buffered solution containing protein stabilizer and $\leq 0.05\%$ ProClin 300
Storage conditions	-20°C

#### 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<sup>1</sup> and restrictively expressed in both normal and malignant plasma cells at high levels<sup>2</sup>. 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.  $2 \times 10^5$  cells were stained for the expression of anti-BCMA CAR with Rabbit Anti-Mouse C11D5.3 scFv Polyclonal Antibody, Biotin (Product No. 500514, 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  $\mu$ L Rabbit Anti-Mouse C11D5.3 scFv Polyclonal Antibody, Biotin into the bottom of the tube.
2. 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.
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  $\mu$ L FACS buffer to the tube. Mix well and centrifuge at 300g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
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  $1 \times 10^6$  nucleated cells per 100  $\mu$ L of buffer.
4. Add 1  $\mu$ L Rabbit Anti-Mouse C11D5.3 scFv Polyclonal Antibody, Biotin. Mix gently and thoroughly.
5. Incubate for 25 minutes at room temperature (18-25°C).
6. 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.
7. Repeat step 6 twice. Then add 100  $\mu$ L 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  $\mu$ 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.
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.

### 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>.