Technical Data Sheet

PureBind F(ab')$_2$ Fragment Anti-Leu16 scFv Monoclonal Antibody, Alexa Fluor 647

Product Information	
Product No.	221602
Size	100 tests
Recommended Vol. per Test	1 µL
Antibody Types	Monoclonal Antibody
Antibody Format	F(ab')$_2$ Fragment
Clone	L16-1G12
Immunogen	scFv region of a CD20-specific Mouse mAb clone Leu16
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 monoclonal antibody L16-1G12 specifically binds to the scFv region of a CD20-specific mouse monoclonal antibody (mAb, clone Leu16). CD20 (cluster of differentiate 20) is a protein that is expressed on the surface of B cells, starting at the pre-B cell stage and also on mature B cells in the bone marrow and in the periphery. CD20 is not expressed on hematopoietic stem cells, pro-B cells, or normal plasma cells. The scFv region of Leu16 has been used to develop CD20-specific chimeric antigen receptor (CAR) T cells utilized in clinical trials.

Preparation & Storage

- Store undiluted at 2-8°C.
- Avoid prolonged exposure to light.
- Avoid freeze/thaw cycle of the reagent.
- The monoclonal 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

FACS Protocol

(Optional) For Whole Blood Sample

1. Pipette 1 µL PureBind F(ab')2 Fragment Anti-Leu16 scFv Monoclonal 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 PureBind F(ab')2 Fragment Anti-Leu16 Monoclonal 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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