

## Technical Data Sheet

### Anti-MSLN (M5 scFv) CAR Idiotype Antibody, Alexa Fluor 647

Product Information	
Product No.	219501
Size	25 tests
Recommended Vol. per Test	1 $\mu$ L
Antibody Types	Recombinant Monoclonal Antibodies
Antibody Format	Whole IgG
Clone	M5-B6
Immunogen	scFv region of a MSLN-specific human mAb clone M5
Conjugate	Alexa Fluor 647
Excitation/Emission Max	651/667nm
Host Species	Rabbit
Storage Buffer	Aqueous buffered solution containing protein stabilizer and $\leq$ 0.05% ProClin 300
Storage conditions	2-8°C, store in dark

#### Description

The rabbit monoclonal antibody M5-B6 specifically binds to the scFv region of a M5-specific human monoclonal antibody (mAb, clone M5). Mesothelin, also known as MSLN, is a glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein. Because of its limited expression in normal tissues and its overexpression in several solid tumors such as ovarian cancer, breast cancer, colorectal cancer and pancreatic cancer, which is associated with a lower survival rate, it is an ideal target for tumor-specific therapy. The scFv region of M5 has been used to develop MSLN 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
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#### FACS Protocol

##### (Optional) For Whole Blood Sample

1. Pipette 1  $\mu$ L Anti-MSLN (M5 scFv) CAR Idiotype 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  $\mu$ 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.

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  $\mu$ L FACS buffer to the tube. Mix well and centrifuge at 300g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
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  $1\times 10^6$  nucleated cells per 100  $\mu$ L of buffer.
4. Add 1  $\mu$ L Anti-MSLN (M5 scFv) CAR Idiotype Antibody, Alexa Fluor 647, dead cell staining solution and additional fluorochrome. Mix gently and thoroughly.

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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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