

## Technical Data Sheet

### Rabbit Anti-Mouse 14G2a Monoclonal Antibody, Alexa Fluor 647

Product Information	
Product No.	220601
Size	25 tests
Recommended Vol. per Test	1 $\mu$ L
Antibody Types	Monoclonal
Antibody Format	Whole IgG
Clone	14B06
Immunogen	scFv region of a GD2-specific mouse mAb clone 14G2a
Conjugate	Alexa Fluor 647
Excitation/Emission Max	651/667nm
Host Species	Rabbit
Reactivity	Mouse
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 14B06 specifically binds to the scFv region of a GD2-specific mouse monoclonal antibody (mAb, clone 14G2a). Disialoganglioside GD2 is a glycosphingolipid highly expressed on neuroblastoma, melanoma, and other tumors of neuroectodermal origin, with limited expression in normal tissues. The scFv region of 14G2a has been used to develop GD2-specific chimeric antigen receptor (CAR) T cells utilized in preclinical studies and 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 Rabbit Anti-Mouse 14G2a 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  $\mu$ 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 300 $\times$ 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.

##### (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<sup>6</sup> nucleated cells per 100  $\mu$ L of buffer.
4. Add 1  $\mu$ L Rabbit Anti-Mouse 14G2a 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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