For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

CD61

Code No.	Clone	Subclass	Quantity	Concentration
D281-3	T74	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Glycoprotein IIIa, a β_3 (CD61) subunit of integrin $\alpha_{IIb}\beta_3$ (CD41/CD61), acts as a fibrinogen receptor on the platelet surface. A mouse monoclonal antibody, T74, recognizes human β_3 (CD61) subunit and inhibits platelet aggregation induced by several agonists such as collagen, ADP, and thrombin. T74 inhibits clot-retraction mediated by platelets or $\alpha_v\beta_3$-expressing cells when induced by thrombin. Western-blot analysis is not possible. T74 fails to bind to platelets from patients with Glanzmann's thrombasthenia.

SOURCE: This antibody was purified from hybridoma (clone T74) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with human platelet.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: It is reported that this clone (T74) recognizes human CD61 antigen in the reference number 1).

APPLICATIONS:
Western blotting; Not recommended
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 1 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:
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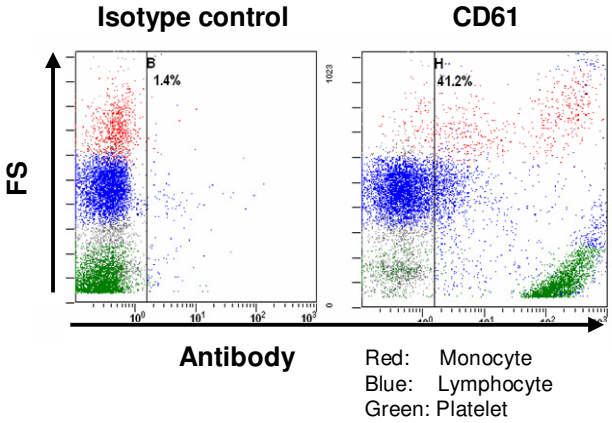
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Platelet Monocyte	Not Tested	Not Tested
Reactivity on FCM	+		

REFERENCES:
1) Katagiri, Y., *et al.*, *J. Biol. Chem.* **270**, 1785-1790 (1995)
2) Prandini, M. H., *et al.*, *Biochem. Biophys. Res. Commun.* **156**, 595-601 (1988)
3) Zimrin, A. B., *et al.*, *J. Clin. Invest.* **81**, 1470-1475 (1988)
4) Kunicki, T. J., *et al.*, *J. Clin. Invest.* **67**, 717-724 (1981)

Clone T74 is used in the reference number 1).



Flow cytometric analysis of CD61 expression on platelet and monocyte. *The staining intensity of D281-3 is shown in the horizontal axis with Forward Scatter intensity on the vertical axis.*

PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Wash the cells 3 times with washing buffer [PBS containing 0.5% BSA and 2 mM EDTA].

2) Resuspend the cells with washing buffer ($4x10^6$ cells/mL).
3) Add 100 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 50 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at 4°C.
6) Wash the cells 3 times with washing buffer.
7) Add 50 µL of 1:200 PE conjugated anti-mouse IgG (MBL; code no. IM-0855) diluted with the washing buffer. Mix well and incubate for 30 minutes at 4°C.
8) Wash the cells 3 times with washing buffer.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

RELATED PRODUCTS:

D274-3	CD42b (TM60)
D274-A48	CD42b-Alexa Fluor®488 (TM60)
D274-A64	CD42b-Alexa Fluor®647 (TM60)
D276-3	CD36 (GS95)
D276-A48	CD36-Alexa Fluor®488 (GS95)
D276-A64	CD36-Alexa Fluor®647 (GS95)
D280-3	CD62P (2T60)
D280-A48	CD62P-Alexa Fluor®488 (2T60)
D280-A64	CD62P-Alexa Fluor®647 (2T60)
D281-3	CD61 (T74)
D281-A48	CD61-Alexa Fluor®488 (T74)
D281-A64	CD61-Alexa Fluor®647 (T74)
PD033	Anti-β1-Tubulin (polyclonal)
M109-3	Mouse CD61 (1-55-4)
IM-0409	CD42b (SZ2)
IM-0648	CD42b-FITC (SZ2)
IM-1417	CD42b-PE (SZ2)
IM-2116	CD61 (SZ21)
IM-0540	CD61 (SZ21)
IM-1758	CD61-FITC (SZ21)
IM-3605	CD61-PE (SZ21)
IM-3716	CD61-PC7 (SZ21)
IM-0765	CD36 (FA6.152)
IM-0766	CD36-FITC (FA6.152)
IM-1315	CD62P (CLBThromb/6)
A07790	CD62P-FITC (CLBThromb/6)
IM-1759	CD62P-PE (CLBThromb/6)
MTG-001	Clear Back
M075-3	Mouse IgG1 isotype control (2E12)
M075-A48	Mouse IgG1 isotype control-Alexa Fluor® 488 (2E12)
M075-A64	Mouse IgG1 isotype control-Alexa Fluor® 647 (2E12)
M076-3	Mouse IgG2a isotype control (6H3)
M076-A48	Mouse IgG2a isotype control-Alexa Fluor® 488 (6H3)
M076-A64	Mouse IgG2a isotype control-Alexa Fluor® 647 (6H3)