

MONOCLONAL ANTIBODY

CD42b

Code No.	Clone	Subclass	Quantity	Concentration
D274-3	TM60	Mouse IgG2a κ	100 μ L	1 mg/mL

BACKGROUND: The platelet glycoprotein GPIb/IX/V complex functions as a receptor for the von Willebrand factor (vWf) and thrombin. The GPIb/IX/V complex comprises GPIb α , GPIb β , GPIX (CD42a), and GPV (CD42d) in the ratio 2:2:2:1. GPIb (CD42b/c) is a heterodimer, composed of GPIb α (CD42b) and GPIb β (CD42c), covalently associated by a disulfide bond. A monoclonal antibody, TM60, specifically recognizes human GPIb α (CD42b) and inhibits ristocetin-induced platelet aggregation and vWf-binding. It also inhibits thrombin-induced platelet aggregation. TM60 fails to bind to platelets from patients with Bernard-Soulier syndrome. The recognition epitope of TM60 is conformational; thus, it is impossible to use for western blot analysis.

SOURCE: This antibody was purified from hybridoma (clone TM60) 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 μ g IgG in 100 μ L 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 (TM60) recognizes human CD42b antigen in the reference number 4).

APPLICATIONS:

- Western blotting; Not recommended
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; 1 μ g/mL (final concentration)

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

INTENDED USE:

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

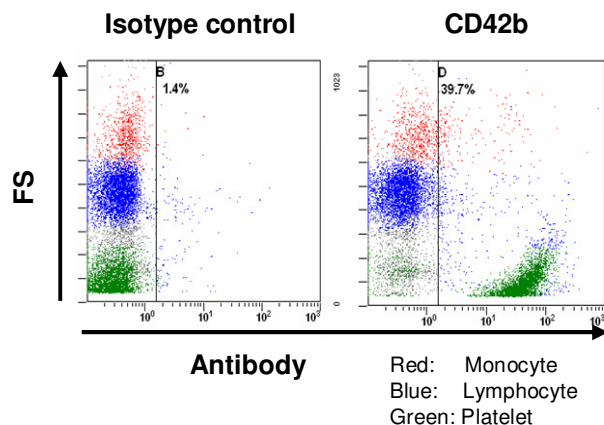
SPECIES CROSS REACTIVITY:

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

REFERENCES:

- 1) Cauwenberghs, N., *et al.*, *Blood* **98**, 652-660 (2001)
- 2) Greco, N. J., *et al.*, *Biochemistry* **35**, 906-914 (1996)
- 3) Arai, M., *et al.*, *Br. J. Haematol.* **87**, 185-188 (1994)
- 4) Yamamoto, N., *et al.*, *Thromb. Res.* **39**, 751-759 (1985)

Clone TM60 is used in the reference number 3) and 4).



Flow cytometric analysis of CD42b expression on platelet and monocyte.
The staining intensity of D274-3 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 (4x10⁶ cells/mL).

- 3) Add 100 μ L 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 μ L 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 μ L 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 μ L 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 μ L of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; human platelet and human monocyte)

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- β 1-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)