

MONOCLONAL ANTIBODY

CD62P

Code No.	Clone	Subclass	Quantity	Concentration
D280-3	2T60	Mouse IgG1 κ	100 μ L	1 mg/mL

BACKGROUND: CD62P, a platelet granule membrane protein (GMP-140), is a member of the selectin family. Since CD62P is expressed by the platelet surface on platelet activation, it is also known as the platelet activation-dependent granule-external membrane protein (PADGEM). CD62P interacts with PSGL-1 (CD162), which mediates the rolling of leukocytes on the surface of activated endothelial cells, the first step in leukocyte extravasation and migration towards inflammations. A monoclonal antibody, 2T60, recognizes both human and rabbit CD62P. In experimental cerebral thrombosis of rabbits, 2T60 detects activated platelets adhering to the damaged subendothelium and thrombi.

SOURCE: This antibody was purified from hybridoma (clone 2T60) 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 (2T60) recognizes human CD62P antigen in the reference number 2).

APPLICATIONS:

Western blotting; Not tested

*It is reported that 2T60 can be used for Western blotting under non reducing condition in the reference number 2).

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:

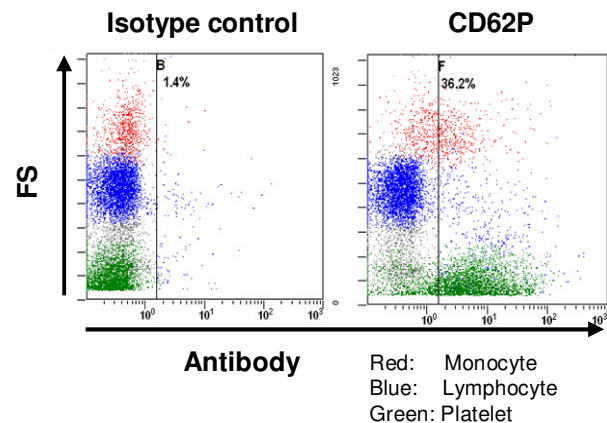
Species	Human	Mouse	Rat
Cells	Platelet Monocyte	Not Tested	Not Tested
Reactivity on FCM	+		

Reactivity of D280-3 to rabbit platelet is not confirmed in our laboratory. However, it is reported that this clone reacts with rabbit platelet in the reference number 2).

REFERENCES:

- 1) Burger, P. C., *et al.*, *Blood* **101**, 2661-2666 (2003)
- 2) Tanoue, K., *et al.*, *Platelets* **4**, 31-39 (1993)
- 3) McEver, R. P., *et al.*, *J. Clin. Invest.* **84**, 92-99 (1989)
- 4) Johnston, G. I., *et al.*, *Cell* **56**, 1033-1044 (1989)

Clone 2T60 is used in the reference number 2).



Flow cytometric analysis of CD62P expression on platelet and monocyte.
The staining intensity of D280-3 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 (4×10^6 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)