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

POLYCLONAL ANTIBODY

Anti-β1-Tubulin

Code No.	Quantity	Form
PD033	100 μL	Affinity Purified

BACKGROUND: Microtubules are one of the components of the cytoskeleton, which perform essential and diverse functions within eukaryotic cells. Microtubules are composed of a heterodimer of α and β tubulins. β1-tubulin is specifically expressed in megakaryocytes and platelets and is required for optimal platelet assembly. It has been reported that the mutation of β1-tubulin gene associated with congenital macrothrombocytopenia affects microtubule assembly.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the KLH conjugated synthetic peptide CKAVLEEDEEVTEEAEMEPEDKGH.

FORMULATION: 100 μL volume of PBS containing 50% glycerol, pH 7.2. Contains no preservative.

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

REACTIVITY: This antibody reacts with human β1-Tubulin but not to β2-, β3-, β4-, β5-, β6-, β8-Tubulin on Western blotting and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1:1,000 for a chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; 1:2,000
Flow cytometry; Not tested

Detailed procedures are provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	platelet, transfectant	Not Tested	Not Tested
Reactivity on WB	+		

REFERENCES:

1) Kunishima, S., *et al., Blood* **113**, 458-461 (2009)
2) Lecine, P., *et al., Blood* **96**, 1366-1373 (2000)

This antibody is used in the reference number 1).

INTENDED USE:

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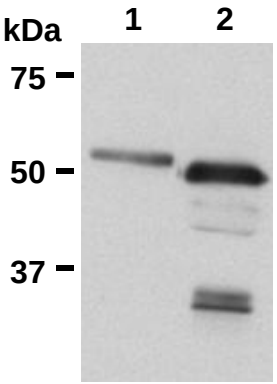


Western blot analysis of β1-Tubulin expression on transfectant (1) and normal human platelets (2) using PD033.

PROTOCOLS:

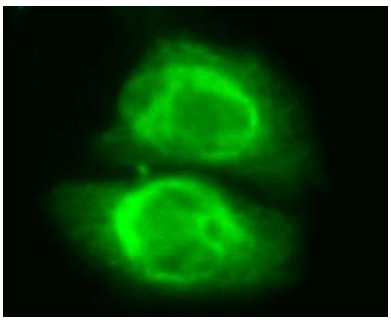
SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 μL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in

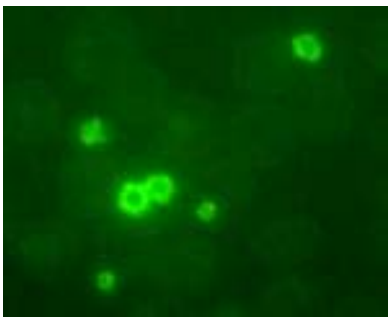
PBS] (5 minutes x 3 times).
7) Incubate the membrane with 1:10,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; transfectant, platelet)

transfectant



platelet



Immunocytochemical detection of β1-Tubulin in platelets on normal human blood and transfectant with PD033.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the glass slide 2 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
4) Wash the glass slide 3 times with PBS.
5) Immerse the slide in PBS containing 0.3% Triton X-100 for 10 minutes at room temperature.
6) Wash the glass slide 3 times with PBS.
7) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) Wash the glass slide 3 times with PBS.
9) Add 200 μL of 1:500 Alexa Fluor® 488 conjugated anti-rabbit IgG (Invitrogen; code no. A110374) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) Wash the glass slide 3 times with PBS.
11) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; transfectant)

Immunocytochemistry for whole blood

1) Prepare the whole blood smear slides.
2) Immerse the slide in 100% methanol to fix the cells for 10 minutes at room temperature.
3) Immerse the slide in 100% acetone to permeabilize the cells for 10 minutes at room temperature.
4) Wash the slide 2 times with PBS.
5) Immerse the slide in normal goat serum for 10 minutes at room temperature.
6) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
7) Wash the slide 2 times with PBS.
8) Add 200 μL of 1:500 Alexa Fluor® 488 conjugated anti-rabbit IgG (Invitrogen; code no. A110374) diluted with PBS onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
9) Wash the glass slide 2 times with PBS.
10) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
11) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; platelet)