

POLYCLONAL ANTIBODY

Loading Control Antibody

## Anti- $\alpha$ -Tubulin pAb

Code No.  
PM054

Quantity  
100  $\mu$ L

Form  
Affinity Purified

**BACKGROUND:** Microtubules are one of the components of the cytoskeleton, which performs essential and diverse functions within eukaryotic cells. Microtubules are composed of a heterodimer of  $\alpha$  and  $\beta$  tubulins. Tubulin is a GTP-binding protein, and extension and shortening of the microtubules are regulated by binding/hydrolysis of GTP.

**SOURCE:** This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with KLH conjugated synthetic peptide, corresponding to N-terminus of  $\alpha$ -tubulin.

**FORMULATION:** 100  $\mu$ 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with human  $\alpha$ -tubulin on western blotting and Immunoprecipitation, and with mouse  $\alpha$ -tubulin on Immunocytochemistry. The reactivity to rat, hamster and chicken  $\alpha$ -tubulin was confirmed by Western blotting.

### APPLICATIONS:

Western blotting; 1:1,000

Immunoprecipitation; 2  $\mu$ L/200  $\mu$ L of cell extract from  $2 \times 10^6$  cells

Immunohistochemistry; Not tested

Immunocytochemistry; 1:200

Flow cytometry; Not tested

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

### SPECIES CROSS REACTIVITY:

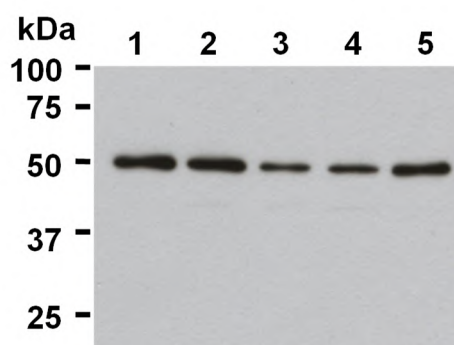
Species	Human	Mouse	Rat	Hamster	Chicken
Cells	HeLa,	NIH/3T3	PC12	CHO	MuH1
Reactivity on WB	+	+	+	+	+

### INTENDED USE:

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

### REFERENCES:

- 1) Makise, M., *et al.*, *BMC Cancer* **18**, 519 (2018) [WB]
- 2) Zhu, K., *et al.*, *Cell Death Dis.* **9**, 500 (2018) [WB]
- 3) Fu, W., *et al.*, *Cell Death Dis.* **8**, e3086 (2017) [WB]
- 4) Murakami, A., *et al.*, *J. Biol. Chem.* **292**, 19976-19986 (2017) [WB]
- 5) Shibutani, M., *et al.*, *Int. J. Mol. Sci.* **18**, E1872 (2017) [WB]
- 6) Xu, D., *et al.*, *Genes Dev.* **31**, 1024-1035 (2017) [WB]
- 7) Ma, K., *et al.*, *Autophagy* **13**, 579-591 (2017) [IC]
- 8) Li, H., *et al.*, *DNA Res.* **23**, 571-580 (2016) [WB]
- 9) Wang, H., *et al.*, *Development* **143**, 530-539 (2016) [WB]
- 10) Xu, D., *et al.*, *Elife* **4**, e10510 (2015) [WB]
- 11) Chinen, T., *et al.*, *Nat. Commun.* **6**, 8722 (2015) [IC]
- 12) Xu, D., *et al.*, *Autophagy* **11**, 617-628 (2015) [WB]
- 13) Horii, T., *et al.*, *Sci. Rep.* **5**, 89076 (2015) [WB]
- 14) Taniuchi, K., *et al.*, *Neoplasia* **16**, 1082-1093 (2014) [IC]
- 15) Cao, J. X., *et al.*, *Cell Death Dis.* **5**, e1426 (2014) [WB]
- 16) Geng, Y., *et al.*, *J. Biol. Chem.* **287**, 30729-30742 (2012) [WB]
- 17) Hall, J. L., and Cowan, N. J., *Nucleic Acids Res.* **13**, 207-223 (1985)



**Western blotting analysis of  $\alpha$ -tubulin in cell lysates from HeLa (1), NIH/3T3 (2), PC12 (3), CHO (4) and MuH1 (5) using PM054.**

Sample volume: 1  $\mu$ g per lane

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

### PROTOCOLS:

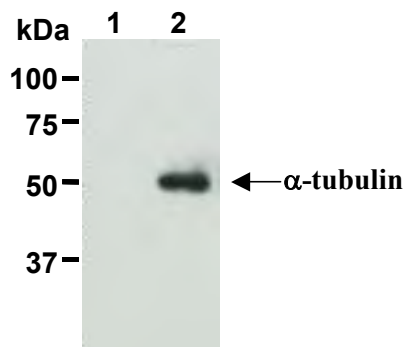
#### **SDS-PAGE & Western blotting**

- 1) Wash cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and resuspend them in 10 volumes of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% NP-40] containing protease inhibitors at appropriate concentrations. Incubate it at  $4^{\circ}\text{C}$  with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10

seconds).

- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 0.2 mg/mL solution.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis
- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
- 6) 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.
- 7) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
- 9) Incubate the membrane with 1:10,000 anti-IgG (Rabbit) pAb-HRP (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (10 minutes x 3).
- 11) Wipe excess buffer off the membrane, and incubate membrane with appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 13) 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; HeLa, NIH/3T3, PC12, CHO and MuH1)

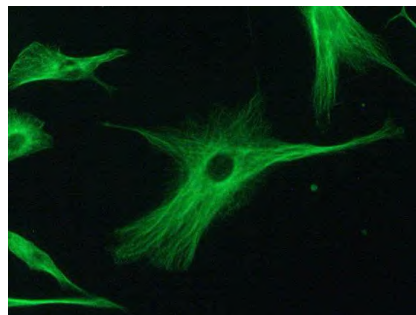


**Immunoprecipitation of  $\alpha$ -tubulin from HeLa with Normal rabbit IgG (1) or PM054 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM054. Rabbit TrueBlot™ (e-Bioscience) is used for secondary antibody.**

### **Immunoprecipitation**

- 1) Wash cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40]. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
- 5) Resuspend the beads with cold Lysis buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
- 7) Repeat steps 5)-6) 2-4 times.
- 8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)



**Immunocytochemical detection of  $\alpha$ -tubulin in NIH/3T3 with PM054.**

### **Immunocytochemistry**

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread  $1 \times 10^4$  cells for one slide, then incubate in a CO<sub>2</sub> incubator overnight.)
- 2) Wash the glass slide twice 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 0.2% Triton X-100 for 10 minutes at room temperature.
- 6) Wash the glass slide twice with PBS.

- 7) Add the primary antibody diluted with PBS containing 2% FCS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 8) Wash the glass slide twice with PBS.
- 9) Add 100  $\mu$ L of 1:500 Alexa Fluor<sup>®</sup> 488 conjugated anti-rabbit IgG (Thermo Fisher Scientific, code no. A11008) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 10) Wash the glass slide twice 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; NIH/3T3)

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