POLYCLONAL ANTIBODY

Anti-α-Tubulin pAb

Code No. PM054

Quantity 100 μL

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 α and β 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 α-tubulin.

FORMULATION: 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: This antibody reacts with human α-tubulin on western blotting and Immunoprecipitation, and with mouse α-tubulin on Immunocytochemistry. The reactivity to rat, hamster and chicken α-tubulin was confirmed by Western blotting.

APPLICATIONS:
Western blotting: 1:1,000 for a chemiluminescence detection system
Immunoprecipitation: 2 μL/200 μL of cell extract from 2 x 10⁶ cells
Immunohistochemistry: Not tested
Immunocytochemistry: 1:200
Flow cytometry: Not tested

Detailed procedures are provided in the following PROTOCOLS.

SPECIES CROSS REACTIVITY:

<table>
<thead>
<tr>
<th>Species</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
<th>Hamster</th>
<th>Chicken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>HeLa, NIH/3T3</td>
<td>PC12</td>
<td>CHO</td>
<td>MuH1</td>
<td></td>
</tr>
<tr>
<td>Reactivity on WB</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


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]
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]
14) Tanouchi, K., et al., Neoplasia 16, 1082-1093 (2014) [IC]

PROTOCOLS:
SDS-PAGE & Western Blotting
1) Wash cells (approximately 1 x 10⁶ 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°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).

Western blot analysis of α-tubulin in cell lysates from HeLa (1), NIH/3T3 (2), PC12 (3), CHO (4) and MuH1 (5) using PM054. Sample volume: 1 µg per lane

Formulation:
50
75
100
25
37
50
25
100
75

kDa

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
E-mail support@mbl.co.jp, TEL 052-238-1904
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/cm² 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.
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 times).
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 times).
11) Wipe excess buffer off the membrane, and incubate 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**

1) Wash cells (approximately 1 x 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) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) 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)

**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 CO2 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 0.2% Triton X-100 for 10 minutes at room temperature.
6) Wash the glass slide 2 times 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.)

**Immunocytochemical detection of α-tubulin in NIH/3T3 with PM054.**

**Immunoprecipitation of α-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 TrueBlotTM (e-Bioscience) is used for secondary antibody.**
8) Wash the glass slide 2 times with PBS.
9) Add 100 µL of 1:500 Alexa Fluor® 488 conjugated anti-rabbit IgG (Invitrogen; 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 2 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; NIH/3T3)

RELATED PRODUCTS:

Loading control antibodies
PM054-7 Anti-α-Tubulin pAb-HRP-DirecT
M175-3 Anti-α-Tubulin mAb (2F9)
PM053 Anti-β-Actin pAb
PM053-7 Anti-β-Actin pAb-HRP-DirecT
M177-3 Anti-β-Actin mAb (6D1)
M171-3 Anti-GAPDH mAb (3H12)
M171-7 Anti-GAPDH mAb-HRP-DirecT (3H12)
PM088 Anti-Vinculin pAb

Organelle specific antibodies
M175-A48 Anti-α-Tubulin mAb-Alexa Fluor® 488 (2F9)
M175-A59 Anti-α-Tubulin mAb-Alexa Fluor® 594 (2F9)
M175-A64 Anti-α-Tubulin mAb-Alexa Fluor® 647 (2F9)
PM062 Anti-EEA1 pAb
M176-3 Anti-EEA1 mAb (3C10)
M176-A48 Anti-EIA1 mAb-Alexa Fluor® 488 (3C10)
M176-A59 Anti-EIA1 mAb-Alexa Fluor® 594 (3C10)
M176-A64 Anti-EIA1 mAb-Alexa Fluor® 647 (3C10)
PM060 Anti-Calnexin pAb
M178-3 Anti-Calnexin mAb (4F10)
M178-A48 Anti-Calnexin mAb-Alexa Fluor® 488 (4F10)
M178-A59 Anti-Calnexin mAb-Alexa Fluor® 594 (4F10)
M178-A64 Anti-Calnexin mAb-Alexa Fluor® 647 (4F10)
PM059 Anti-KDEL pAb
M181-3 Anti-KDEL mAb (1D5)
PM061 Anti-GM130 pAb
M179-3 Anti-GM130 mAb (5G8)
M179-A48 Anti-GM130 mAb-Alexa Fluor® 488 (5G8)
M179-A59 Anti-GM130 mAb-Alexa Fluor® 594 (5G8)
M179-A64 Anti-GM130 mAb-Alexa Fluor® 647 (5G8)
PM063 Anti-COX4 pAb
PM064 Anti-Lamin B1 pAb
PD030 Anti-CENP-C (Human) pAb
D282-3 Anti-CENP-K (ICEN37) (Human) mAb (46F1)
PD018 Anti-CENP-K (ICEN37) (Human) pAb
PM036 Anti-LC3 pAb
M152-3 Anti-LC3 mAb (4E12)

Other related antibodies and kits are also available.
Please visit our website at [http://ruo.mbl.co.jp/](http://ruo.mbl.co.jp/)