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MONOCLONAL ANTIBODY **Loading Control Antibody**

Anti-α-Tubulin mAb

Code No.	Clone	Subclass	Quantity	Concentration
M175-3	2F9	Mouse IgG2a κ	100 µL	2 mg/mL

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 hybridoma (clone 2F9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the synthetic peptide corresponding to N-terminal of human α-Tubulin.

FORMULATION: 200 µg IgG in 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, Immunoprecipitation, and Immunocytochemistry. The reactivity to mouse, rat, hamster and chicken α-tubulin was confirmed by Western blotting.

APPLICATIONS:

Western blotting; 2 µg/mL
Immunoprecipitation; 5 µg/200 µL of cell extract from 2 x 10^6 cells
Immunocytochemistry; 2 µg/mL
Immunohistochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

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

REFERENCES:

1) Li, T., *et al*., *Cell Death. Dis*. **5**, e1229 (2014) [WB]
2) Hino, K., *et al*., *J. Virol*. **87**, 6582-6588 (2013) [WB]
3) Zhang, S., *et al*., *Biochem. Biophys. Res. Commun*. **427**, 537-541 (2012) [WB]
4) Heald, R., and Nogales, E., *J. Cell Sci*. **115**, 3-4 (2002)
5) Hall, J. L., and Cowan, N. J., *Nucleic Acids Res.* **13**, 207-223 (1985)

INTENDED USE:

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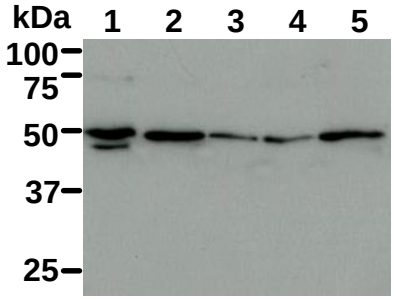


Western blotting analysis of α-Tubulin in HeLa (1), NIH/3T3 (2), PC12 (3), CHO (4) and MuH1 (5) using M175-3.
Sample volume: 2 µg per lane

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

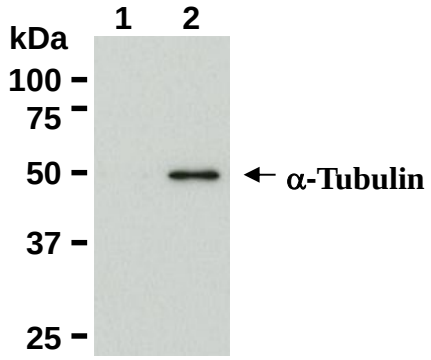
PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 10 volume 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).
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 20 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^2 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 (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 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 5 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, MuH1)

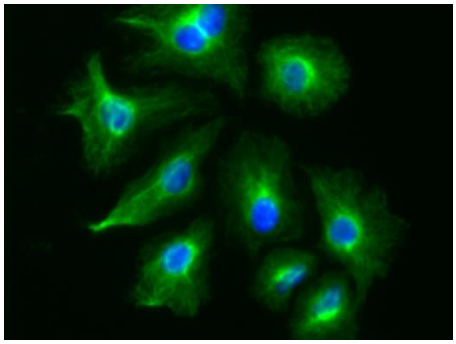


Immunoprecipitation of α-Tubulin from HeLa with mouse IgG2a isotype control (1) or M175-3 (2). *After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with anti-α-Tubulin polyclonal antibody (MBL, code no. PM054).*

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) 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 α-Tubulin in HeLa using M175-3.
Green: anti-α-Tubulin
Blue: DAPI counter stain

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 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 are recommended if necessary.)
8) Wash the glass slide twice with PBS.
9) Add 100 µL of 1:500 Alexa Fluor® 488 conjugated anti-mouse IgG (Thermo Fisher Scientific, code no. A11001) 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) Counter stain with DAPI for 5 minutes at room temperature.

12) Wash the glass slide twice with PBS.
13) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
14) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)

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