

MONOCLONAL ANTIBODY

Loading Control Antibody

Anti- α -Tubulin mAb

Code No.	Clone	Subclass	Quantity	Concentration
M175-3	2F9	Mouse IgG2a κ	100 μ L	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 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: 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 μ g/mL

Immunoprecipitation; 5 μ g/200 μ L of cell extract from 2 x 10⁶ cells

Immunocytochemistry; 2 μ g/mL

Immunohistochemistry; Not tested

Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

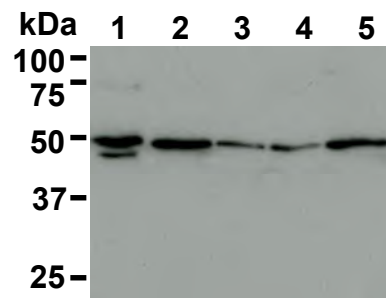
Species	Human	Mouse	Rat	Hamster	Chicken
Cells	HeLa	NIH/3T3	NRK	CHO	MuH1
Reactivity on WB	+	+	+	+	+

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:

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



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 μ g per lane

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

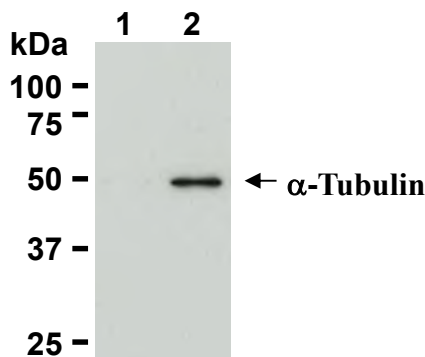
PROTOCOLS:

SDS-PAGE & Western blotting

- 1) Wash cells (approximately 1 x 10⁷ 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 μ L 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% 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).

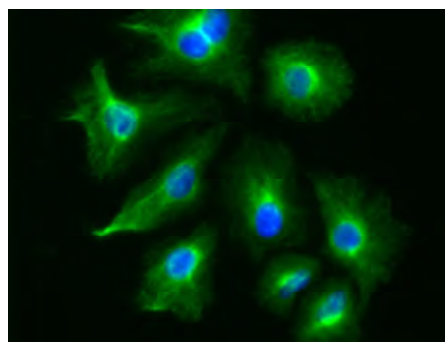
Immunoprecipitation

- 1) Wash cells (approximately 1×10^7 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 μ L of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20 μ L 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 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 μ L/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 1×10^4 cells for one slide, then incubate in a CO₂ 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 μ L 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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