**Anti-α-Tubulin mAb**

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

**REFERENCES:**

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

**SPECIES CROSS REACTIVITY:**

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<th>Rat</th>
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<td>NRK</td>
<td>CHO</td>
<td>MuH1</td>
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<tr>
<td>Reactivity on WB</td>
<td>+</td>
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**APPLICATIONS:**
- Western blotting: 2 μg/mL
- Immunoprecipitation: 5 μg/200 μL of cell extract from 2 x 10^6 cells
- Immunohistochemistry: Not tested
- Immunocytochemistry: 2 μg/mL
- Flow cytometry: Not tested

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

**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.

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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 M1753-3 (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 10⁷ 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 10⁴ 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 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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