

MONOCLONAL ANTIBODY

Loading Control Antibody

Anti-β-Actin mAb

Code No.	Clone	Subclass	Quantity	Concentration
M177-3	6D1	Mouse IgG1 κ	100 μL	1 mg/mL

BACKGROUND: Actin is a 42 kDa protein found in eukaryotic cells. Actin is also one of the most highly conserved proteins. Actin participates in many important cellular activities including muscle contraction, cell movement, cell division and the formation of the cytoskeleton. The beta actin exists in most cell types as components of the cytoskeleton.

SOURCE: This antibody was purified from hybridoma (clone 6D1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with KLH conjugated synthetic peptide, corresponding to N-terminus of β-actin.

FORMULATION: 100 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 β-actin on western blotting and Immunoprecipitation. The reactivity to mouse, rat, hamster and chicken β-actin was confirmed by Western blotting.

APPLICATIONS:

Western blotting; 1 μg/mL

Immunoprecipitation; 2 μg/200 μL of cell extract from 2 x 10⁶ cells

Immunohistochemistry; Not tested

Immunocytochemistry; Not recommended

Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

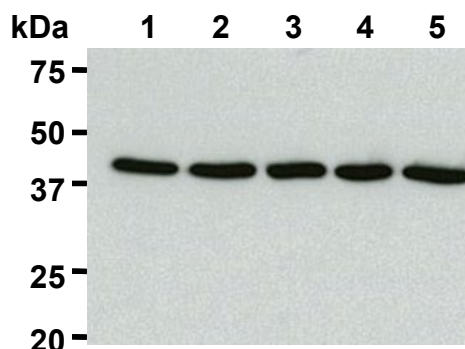
Species	Human	Mouse	Rat	Hamster	Chicken
Cells	HeLa	NIH/3T3	NRK	CHO	MuH1
Reactivity on WB	+	+	+	+	+

INTENDED USE:

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

REFERENCES:

- 1) Tong, L., *et al.*, *Sci. Rep.* **8**, 11525 (2018) [WB]
- 2) Ohkuro, M., *et al.*, *Nat. Commun.* **9**, 1982 (2018) [WB]
- 3) Hori, I., *et al.*, *Sci. Rep.* **7**, 3552 (2017) [WB]
- 4) Sun, H., *et al.*, *Oncotarget* **7**, 76238-76249 (2016) [WB]
- 5) Xing, M., *et al.*, *Nat. Commun.* **6**, 6233 (2015) [WB]
- 6) Watanabe-Nakayama, T., *et al.*, *Biol. Open* **2**, 667-674 (2013) [WB]
- 7) Vandekerckhove, J. and Weber, K., *PNAS.* **75**, 1106 (1978)



Western blot analysis of β-Actin in HeLa (1), NIH/3T3 (2), NRK (3), CHO (4) and MuH1 (5) using M177-3.
Sample volume: 1 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 10⁷ cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 3 minutes and centrifuge. Load 20 μL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 3) 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.
- 4) 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.
- 5) 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

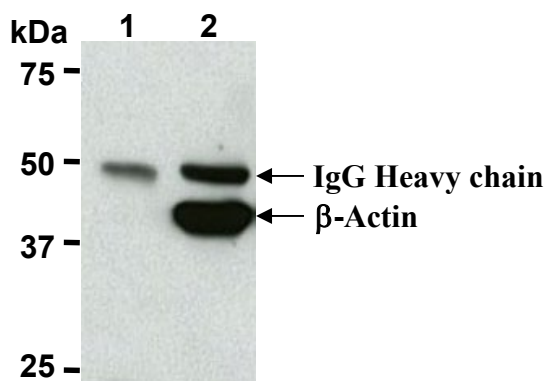
- concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
 - 7) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
 - 8) Wash the membrane with PBS-T (10 minutes x 3 times).
 - 9) Wipe excess buffer off the membrane, and incubate membrane with appropriate chemiluminescence reagent for 1 minute.
 - 10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
 - 11) 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.

- 5) Resuspend the agarose with cold Lysis buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 7) Repeat steps 4)-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)

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Immunoprecipitation of β -Actin from HeLa with mouse IgG1 isotype control, M075-3 (1) or M177-3 (2). After immunoprecipitation with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with M177-3.

(Positive controls for Western blotting; HeLa, NIH/3T3, NRK, CHO and MuH1)

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 discard the supernatant.