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

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Loading Control Antibody

Anti-β-Actin mAb

Code No.	Clone	Subclass	Quantity	Concentration
M177-3MS	6D1	Mouse IgG1 κ	20 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: 20 µg IgG in 20 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 for a chemiluminescence detection system
Immunoprecipitation; 2 µg/200 µL of cell extract from 2 x 10^6 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:
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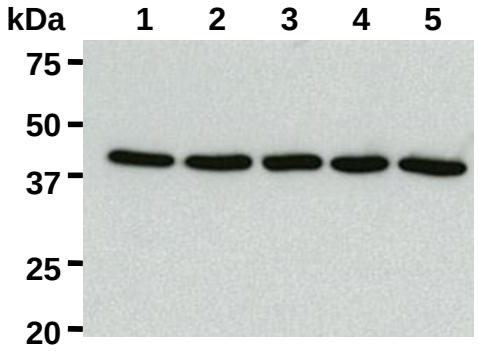
REFERENCE:
Vandekerckhove, J. and Weber, K., *PNAS* **75**, 1106 (1978)



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

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 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/cm^2 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 PBS (pH 7.2) containing 1% skimmed milk 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 HRP-conjugated anti-mouse IgG (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).

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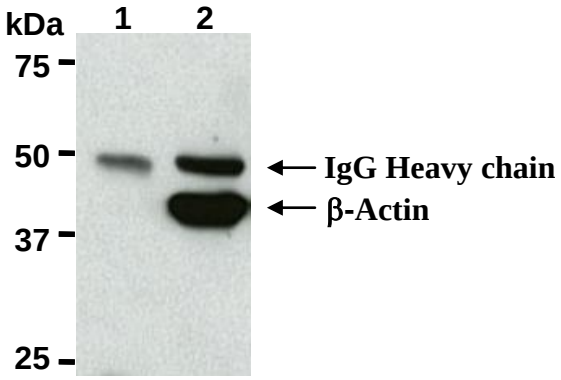
9) Wipe excess buffer off the membrane, and incubate membrane with appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

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



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

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 controls for Immunoprecipitation; HeLa)

RELATED PRODUCTS:

Loading control antibodies

M177-3	Anti-β-Actin mAb
PM053	Anti-β-Actin pAb
PM053-7	Anti-β-Actin pAb-HRP-DirecT
M171-3	Anti-GAPDH mAb
M171-7	Anti-GAPDH mAb-HRP-DirecT
PM054	Anti-α-Tubulin pAb (2F9)
PM054-7	Anti-α-Tubulin pAb-HRP-DirecT
M175-3	Anti-α-Tubulin mAb