

Anti-Myc-tag mAb

CODE No.	M192-3
CLONALITY	Monoclonal
CLONE	My3
ISOTYPE	Mouse IgG2b κ
QUANTITY	200 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, EQKLISEEDL (Myc-tag)
FORMULATION	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.

APPLICATIONS-CONFIRMED

<u>Western blotting</u>	0.1 μ g/mL
<u>Immunoprecipitation</u>	2 μ g/300 μ L of cell extract from 3 x 10 ⁶ cells
<u>Immunocytochemistry</u>	0.5 μ g/mL
<u>Flow cytometry</u>	0.1 μ g/mL

APPLICATION-REPORTED

<u>Chromatin immunoprecipitation</u>	Reference 1)
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REFERENCES

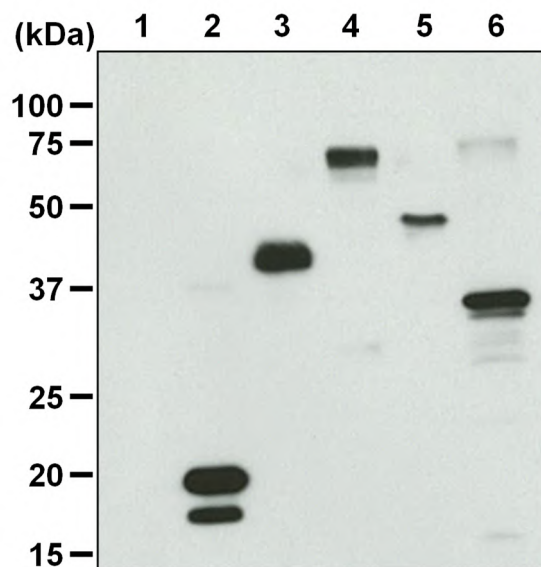
- 1) Chi, Y., *et al.*, *Open Biol.* **8**, 180043 (2018) [ChIP]
- 2) Tu, R., *et al.*, *Cell Death Dis.* **9**, 553 (2018) [WB, IP]
- 3) Deng, T., *et al.*, *PNAS.* **115**, 4678-4683 (2018) [WB, IP]
- 4) Wu, Y., *et al.*, *PLoS One* **13**, e0190407 (2018) [WB, IP]
- 5) Hu, L., *et al.*, *Plant Cell.* **29**, 3157-3185(2017) [WB]
- 6) Wang, S., *et al.*, *Cell Death Dis.* **8**, e3058 (2017) [WB]
- 7) Horibata, Y., *et al.*, *Sci. Rep.* **7**, 8793 (2017) [WB, IC]
- 8) Habata, S., *et al.*, *Int. J. Oncol.* **49**, 402-410 (2016) [WB, Co-IP]
- 9) Masaki, S., *et al.*, *Int. J. Mol. Sci.* **16**, 3705-3721 (2015) [IC]
- 10) Nomura, T., *et al.*, *J. Biol. Chem.* **289**, 1192-1202 (2014) [IC]

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

SDS-PAGE & Western blotting

- 1) Wash 1×10^6 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 15 sec.).
- 2) Boil the samples for 2 min. and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. 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.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS (5 min. x 3).
- 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 hr. at room temperature.
- 8) Wash the membrane with PBS (5 min. x 3).
- 9) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 10) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



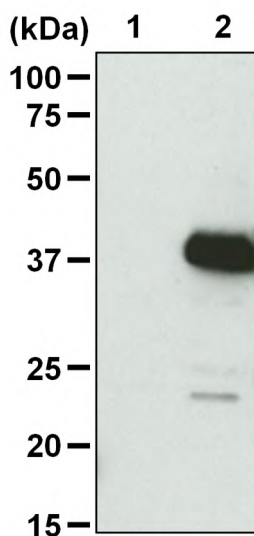
Western blotting analysis of Myc-tagged protein

- Lane 1: Parental cell (293T)
- Lane 2: N-terminal Myc-tagged protein A/293T
- Lane 3: C-terminal Myc-tagged protein B/293T
- Lane 4: C-terminal Myc-tagged protein C/293T
- Lane 5: C-terminal Myc-tagged protein D/293T
- Lane 6: Internal Myc-tagged protein E

Immunoblotted with Anti-Myc-tag mAb (MBL, code no. M192-3)

Immunoprecipitation

- 1) Wash 1×10^7 cells twice with PBS and resuspend them with 1 mL of ice-cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors, then sonicate briefly (up to 20 sec.).
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 300 µL of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gently agitation for 1 hr. at room temperature.
- 4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 5) Resuspend the beads with 1 mL of IP buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 7) Repeat steps 5)-6) twice.
- 8) Add 300 µL of cell lysate (prepared sample from step 2), then incubate with gentle agitation for 1 hr. at room temperature.
- 9) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 10) Resuspend the beads with 1 mL of Lysis buffer.
- 11) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 12) Repeat steps 10)-11) 4 times.
- 13) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 2 min. and centrifuge.
- 14) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 15) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. 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.
- 16) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 17) Incubate the membrane with 1 µg/mL Anti-Myc-tag mAb-HRP-DirecT (MBL, code no. M047-7) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 18) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3).
- 19) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
- 20) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 21) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



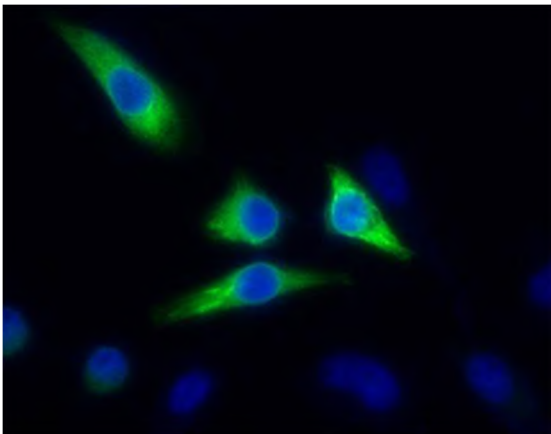
Immunoprecipitation of Myc-tagged protein from transfectant

- Lane 1: IP with Mouse IgG2b (isotype control) (MBL, code no. M077-3)
Lane 2: IP with Anti-Myc-tag mAb (MBL, code no. M192-3)

Immunoblotted with Anti-Myc-tag mAb-HRP-DirecT (MBL, code no. M047-7)

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ incubator overnight.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Fix the cells by immersing the slide in 4% paraformaldehyde/PBS for 10 min. at room temperature (20~25°C).
- 4) Wash the slide twice with PBS.
- 5) Immerse the slide in 0.2% Triton X-100/PBS for 10 min. at room temperature.
- 6) Wash the slide twice with PBS.
- 7) Add 200 µL of the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 8) Wash the slide twice with PBS.
- 9) Add 100 µL of 1:500 anti-IgG (Mouse)-Alexa Fluor® 488 (Thermo Fisher Scientific, code no. A-11001) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
- 10) Wash the slide twice with PBS.
- 11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Counter stain with DAPI for 5 min. at room temperature.
- 13) Wash the slide twice with PBS.
- 14) Promptly add mounting medium onto the slide, then put a cover slip on it.



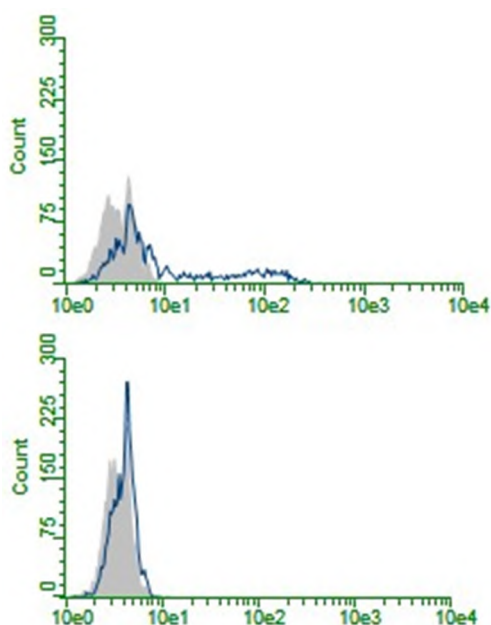
Immunocytochemical detection of Myc-tagged protein in transfectant

Green: Anti-Myc-tag mAb (MBL, code no. M192-3)

Blue: DAPI

Flow cytometric analysis

- 1) Wash the cells (5×10^5 cells/sample) twice with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Add 100 μ L of 4% paraformaldehyde/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
- 3) Wash the cells twice with 1 mL of washing buffer.
- 4) Add 100 μ L of 0.2% Triton X-100 in PBS to the cell pellet after tapping. Mix well, then permeabilize the cells for 10 min. at room temperature.
- 5) Wash the cells once with 1 mL of washing buffer.
- 6) Add 30 μ L of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
- 7) Wash the cells once with 1 mL of washing buffer.
- 8) Add 30 μ L of 1:100 anti-IgG (Mouse)-Alexa Fluor[®] 488 (Thermo Fisher Scientific, code no. A-11001) diluted with the washing buffer. Mix well and incubate for 15 min. at room temperature.
- 9) Wash the cells once with 1 mL of washing buffer.
- 10) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.



Flow cytometric detection of Myc-tagged protein in transfectant

Antibody

Open: Anti-Myc-tag mAb (MBL, code no. M192-3)

Closed: Mouse IgG2b (isotype control) (MBL, code no. M077-3)

Cell

Upper: Myc-tagged protein/HeLa

Lower: Parental cell (HeLa)