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Not for use in diagnostic procedures.

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Anti-Myc-tag mAb

CODE No.	M192-3
CLONALITY	Monoclonal
CLONE	My3
ISOTYPE	Mouse IgG2b κ
QUANTITY	200 µL, 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

Western blotting	0.1 µg/mL
Immunoprecipitation	2 µg/300 µL of cell extract from 3 x 10^6 cells
Immunocytochemistry	0.5 µg/mL
Flow cytometry	0.1 µg/mL

APPLICATION-REPORTED

Chromatin immunoprecipitation Reference 1)

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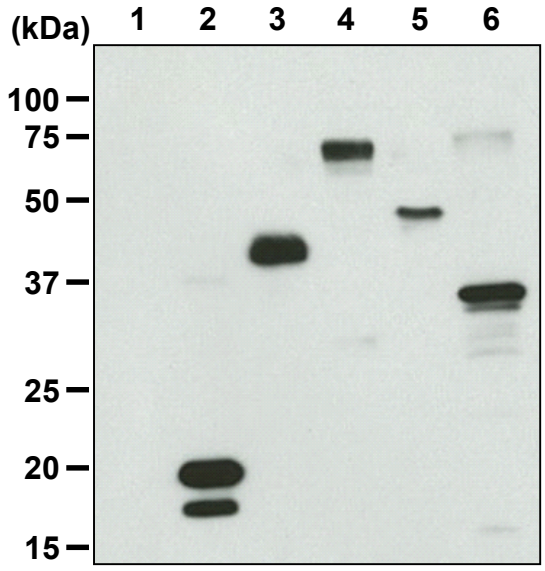
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 1 x 10^6 cells 3 times with PBS and suspends 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 μL 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/cm^2 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.



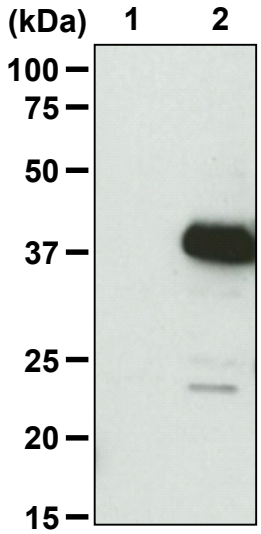
Western blotting analysis of Myc-tagged protein

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 4: Internal Myc-tagged protein E

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

Immunoprecipitation

1) Wash 1 x 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/cm^2 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.



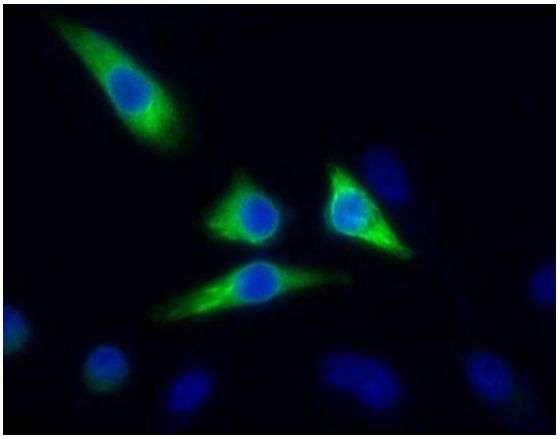
Immunoprecipitation of Myc-tagged protein from transfectant

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 CO_2 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.

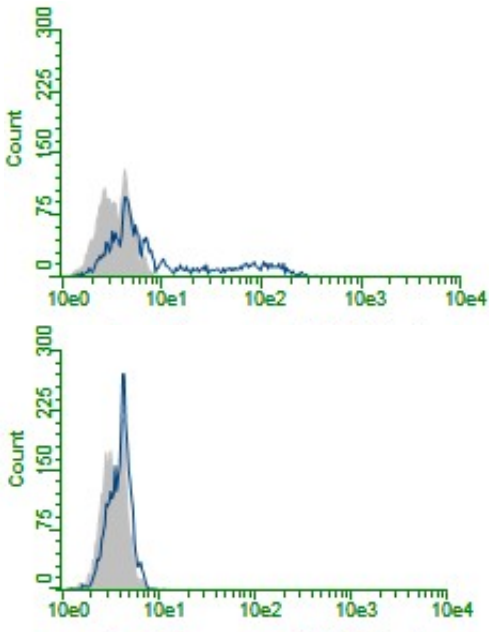


Immunocytochemical detection of Myc-tagged protein in transfectant

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

Flow cytometric analysis

1) Wash the cells (5 x 10^5 cells/sample) twice with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
2) Add 100 µL 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 µL 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 µL 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 µL 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 µL 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)