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

MBL

Anti-HA-tag mAb

CODE No. M180-3

CLONALITY Monoclonal
CLONE TANA2
ISOTYPE Mouse IgG2b κ
QUANTITY 200 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN KLH conjugated synthetic peptide, YPYDVPDYA (HA-tag)
REACTIVITY This antibody reacts with N-terminal and C-terminal HA-tagged proteins.
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 1 µg/mL
Flow cytometry 1 µg/mL

APPLICATION-REORTED
RNP Immunoprecipitation (RIP) Reference 14)

REFERENCES
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5) Tu, R., *et al., Cell Death Dis.* **9**, 553 (2018) [WB, IP]
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7) Lin, C. C., *et al., Sci. Rep.* **8**, 2743 (2018) [WB, IP, IC]
8) Zhang, P., *et al., Nat. Med.* **24**, 84-94 (2018) [WB, IP, IC]
9) Huang, Y., *et al., Oncotarget* **8**, 83075-83087 (2017) [WB, IP]
10) Hu, L., *et al., Plant Cell* **29**, 3157-3185 (2017) [WB]
11) Wang, S., *et al., Cell Death Dis.* **8**, e3058 (2017) [WB]
12) Song, K. H., *et al., Cell Death Dis.* **8**, e2536 (2017) [WB, IP]
13) Iimori, M., *et al., Nat. Commun.* **7**, 11117 (2016) [WB]
14) Zhong, E., *et al., Nucleic Acids Res.* **43**, 10474-10491 (2015) [RIP, Co-IP]
15) Yamano, K., *et al., J. Biol. Chem*. **290**, 25199-15211 (2015) [WB]
16) Okatsu, K., *et al., J. Cell Biol.* **209**, 111-128 (2015) [WB]
17) Iguchi, M., *et al., J. Biol. Chem.* **288**, 22019-22032 (2013) [WB]
18) Tamura, Y., *et al., Cell Metab.* **17**, 709-718 (2013) [WB]

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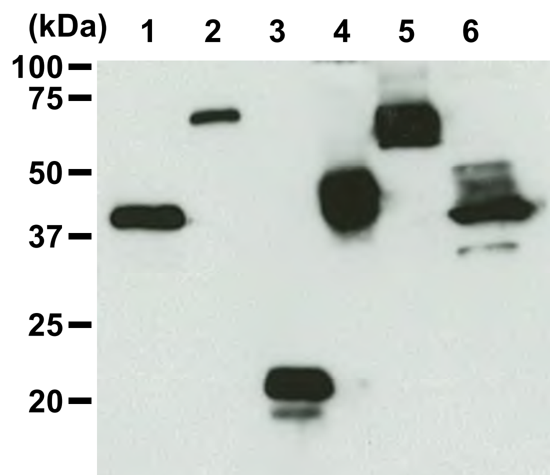
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 10 sec.).
2) Boil the samples for 3 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) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
6) 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.)
7) Wash the membrane with PBS-T (5 min. x 3).
8) Incubate the membrane with 1:10,000 of 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.
9) Wash the membrane with PBS-T (5 min. x 3).
10) 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.
11) Expose to an X-ray film in a dark room for 30 sec. Develop the film as usual. The condition for exposure and development may vary.



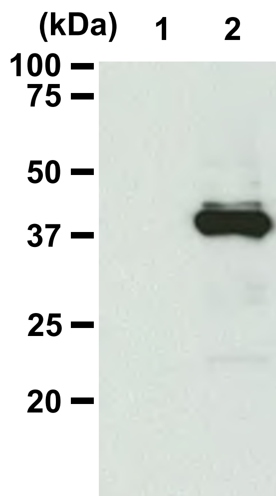
Western blot analysis of HA-tagged protein

Lane 1: N-terminal Met-HA-tagged protein A/293T
Lane 2: N-terminal Met-HA-tagged protein B/293T
Lane 3: N-terminal Met-HA-tagged protein C/293T
Lane 4: N-terminal HA-tagged protein D/293T
Lane 5: N-terminal HA-tagged protein E/293T
Lane 6: C-terminal HA-tagged protein F/293T

Immunoblotted with Anti-HA-tag mAb (MBL, code no. M180-3)

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times 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 gentle agitation for 1 hr. at room temperature.
4) Wash the beads 3 times with 1 mL of IP buffer.
5) Add 300 µL of cell lysate (prepared sample of step 2), then incubate with gentle agitation for 1 hr. at room temperature.
6) Wash the beads 5 times with 1 mL of Lysis buffer.
7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 2 min. and centrifuge.
8) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
9) 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.
10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
11) Incubate the membrane with 1:1,000 of Anti-HA-tag pAb-HRP-DirecT (MBL, code no. 561-7) 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.)
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
13) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
14) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
15) Expose to an X-ray film in a dark room for 30 sec. Develop the film as usual. The condition for exposure and development may vary.



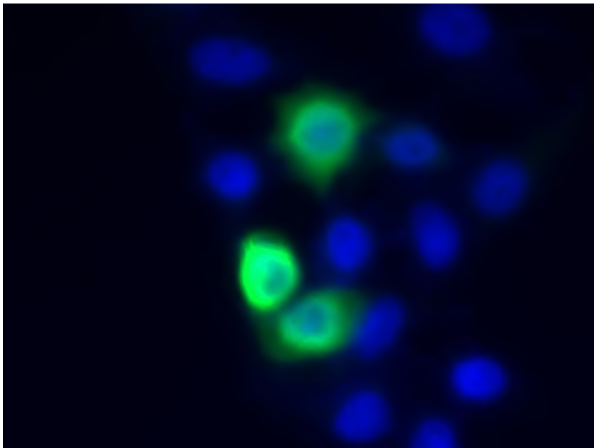
Immunoprecipitation of HA-tagged IκBα

Lane 1: Mouse IgG2b (isotype control) (MBL, code no. M077-3)
Lane 2: Anti-HA-tag mAb (MBL, code no. M180-3)

Immunoblotted with Anti-HA-tag pAb-HRP-DirecT (MBL, code no. 561-7)

Immunocytochemistry

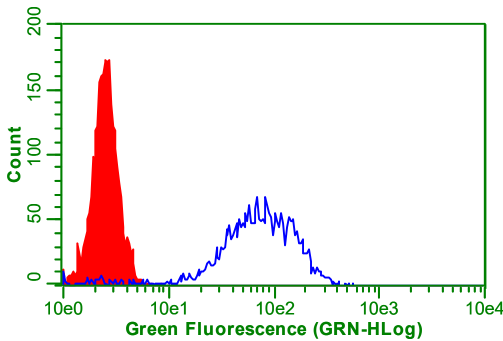
1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
4) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
5) Immerse the slide in 0.2% Triton X-100/PBS for 10 min. at room temperature.
6) Wash the slide in a plenty of PBS as in the step 4).
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 in a plenty of PBS as in the step 4).
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 min. at room temperature. Keep out light by aluminum foil.
10) Wash the slide in a plenty of PBS as in the step 4).
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Counterstain with DAPI for 5 min. at room temperature.
13) Wash the slide in a plenty of PBS as in the step 4).
14) Promptly add mounting medium onto the slide, then put a cover slip on it.

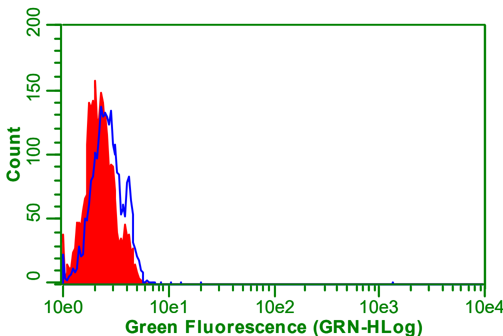


Immunocytochemical detection of HA-tagged IκBα in HeLa
Green: Anti-HA-tag mAb (MBL, code no. M180-3)
Blue: DAPI

Flow cytometric analysis for adherent cells

1) Detach the cells from culture dish.
2) Wash the cells 3 times with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
3) Add 200 µL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
4) Wash the cells 2 times with 1 mL of washing buffer.
5) Add 200 µL of PBS containing 0.2% Triton X-100 to the cell pellet after tapping. Mix well, then permeabilize the cells for 10 min. at room temperature.
6) Wash the cells 1 time with 1 mL of washing buffer.
7) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).
8) Add 100 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 min. at room temperature (20~25°C). Remove supernatant by careful aspiration.
9) Add 20 µL of Clear Back (Human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 min. at room temperature.
10) Add 40 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.
11) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration. Repeat another wash once more.
12) Add 40 µL of 1:500 Alexa Fluor®488 conjugated anti-mouse IgG (Thermo Fisher Scientific, code no. A11001) diluted with washing buffer. Mix well and incubate for 30 min. at room temperature.
13) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
14) Resuspend the cells with 500 µL of washing buffer and analyze by a flow cytometer.





Flow cytometric detection of HA-tagged protein in HeLa

Open: Anti-HA-tag mAb (MBL, code no. M180-3)
Closed: Mouse IgG2b (isotype control) (MBL, code no. M077-3)

Upper: HA-tagged protein in HeLa
Lower: Parental cell (HeLa)