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MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-HA-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M132-3	5D8	Mouse IgG1 κ	100 µL	2 mg/mL

BACKGROUND: Epitope tagging has widely been accepted technique that fuses an epitope peptide to a certain protein as a marker for gene expression. With this technique, the gene expression can be easily monitored on western blotting, immunoprecipitation and immunofluorescence utilizing with an antibody that recognizes such an epitope. Amino acid sequences that are widely used for the epitope tagging are as follow; YPYDVPDYA (HA-tag), EQKLISEEDL (Myc-tag) and YTDIEMNRLGK (VSV-G-tag), which corresponding to the partial peptide of Influenza hemagglutinin protein, Human c-myc gene product and Vesicular stomatitis virus glycoprotein respectively.

SOURCE: This antibody was purified from hybridoma (clone 5D8) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with carrier protein (*CP*) conjugated peptide *CP*-YPYDVPDYA.

FORMULATION: 200 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 N-terminal and C-terminal HA-tag on Western blotting, and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1 µg/mL for a chemiluminescence detection system
Immunoprecipitation; 1 µg/Sample
Immunohistochemistry; Not tested
Immunocytochemistry; Not recommended
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

REFERENCES:

1) Mukai, R. and Ohshima, T., *Mol. Cell. Biol.* **36**, 3075-3085 (2016) [WB, Co-IP]
2) Ogi, T., *et al., PLoS Gent.* **8**, e1002945 (2012) [IP]
3) Kim, T. S., *et al., Dis. Model Mech.* **3**, 752-762 (2010) [WB]

INTENDED USE:

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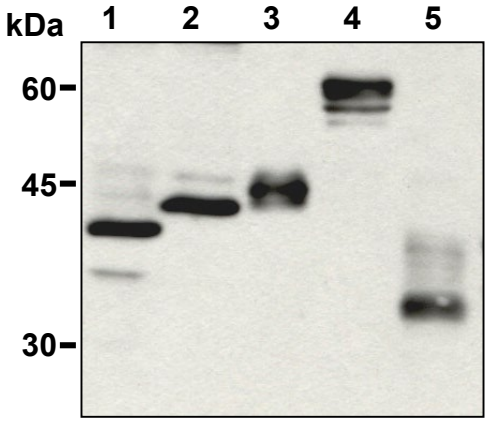
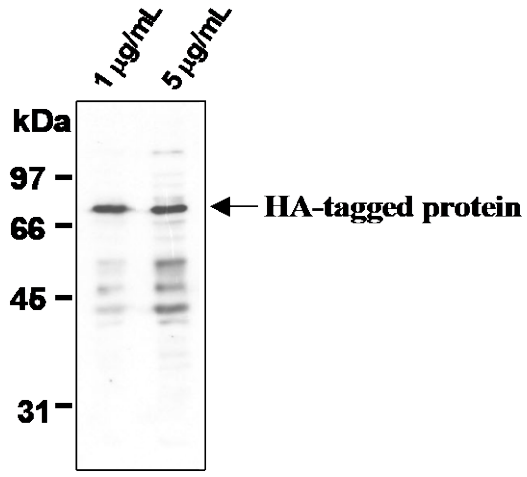


Western blot analysis of C-terminal (1), N-terminal (2) and internal (3, 4, 5) HA-tagged proteins using M132-3.



Westernblot analysis of HA-tagged protein in transfectant using M132-3.

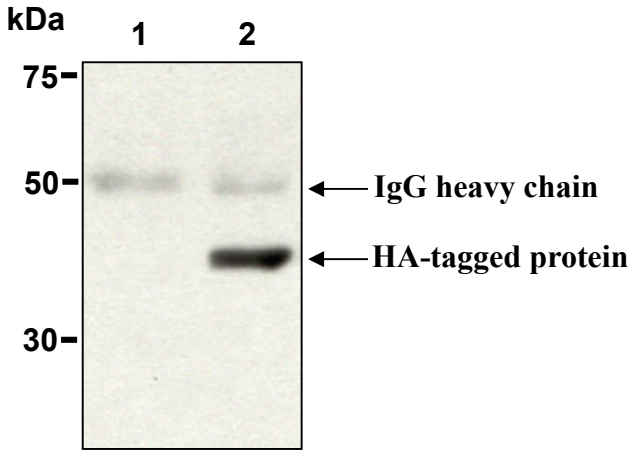
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash cells (approximately 5 x 10^6 cells) 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the 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 5% 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 (5 minutes x 3 times).
9) Wipe excess buffer off the membrane, then incubate the membrane with an appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
10) Expose the membrane onto an X-ray film under usual settings. The conditions for exposure and development may vary.



Immunoprecipitation of HA-tagged transfectant with mouse IgG1 isotype control (MBL; code no. M075-3) (1) or M132-3 (2). *After immunoprecipitation with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-HA-tag polyclonal antibody (MBL; code no.561).*

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 rotation for 30 minutes, then 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 500 µL of cell extract. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 1 hour at 4°C.
5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
6) Resuspend the beads with cold Lysis buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
8) Repeat Step 5)-7) 2-4 times.
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
10) Load 20 µL of the sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
11) 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.
12) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
13) Incubate the membrane with 1:1,000 of Anti-HA-tag pAb (MBL; code no. 561) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
15) Incubate the membrane with 1:10,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
16) Wash the membrane with PBS-T (5 minutes x 3 times).
17) Wipe excess buffer off the membrane, and incubate the membrane with an appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
18) Expose the membrane onto an X-ray film in a dark room for 5 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

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