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MBL
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POLYCLONAL ANTIBODY

Anti-HA-tag pAb-Agarose

Code No.	Quantity
561-8	Gel: 200 µL

BACKGROUND: Epitope tagging has widely been accepted technique that fuse 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 rabbit serum using affinity column. The rabbit was immunized with carrier protein (*CP*) conjugated synthetic peptide, *CP*-YPYDVPDYA (HA-tag).

FORMULATION: 540 µg of anti-HA-tag polyclonal antibody covalently coupled to 200 µL of agarose gel and provided as a 50% gel slurry suspended in PBS containing preservative (0.09% sodium azide) for a total volume of 400 µL.

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody recognizes HA-tag peptide sequence (YPYDVPDYA) on Immunoprecipitation.

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; 20 µL of gel slurry
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

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

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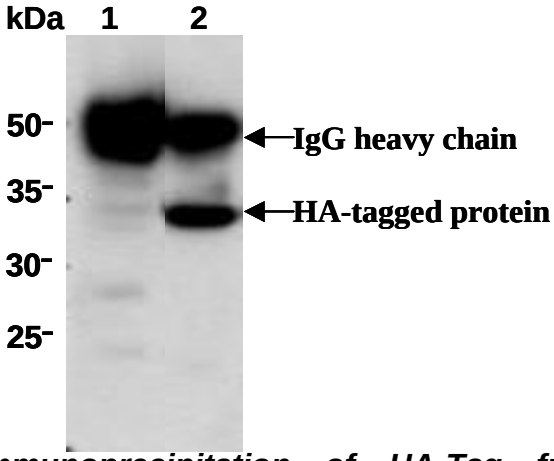
This antibody is used in these references.



Immunoprecipitation of HA-Tag from 293T/pcDNA-HA-tagged protein cell lysate with rabbit IgG (1) and 561-8 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with 561.*

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

PROTOCOL:

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (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 tube.
3) Add agarose as suggest in the **APPLICATIONS** into 200 µL of cell extract. Mix well and incubate with gentle agitation for 30-120 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 agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
6) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
7) 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.
8) 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.
9) Incubate the membrane with 1:1,000 diluted anti-HA-tag polyclonal antibody (MBL; code no. 561) diluted with PBS, pH 7.2 containing 1% skimmed for 1 hour at room temperature. (The concentration of antibody to be used will be depend on condition.)
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
11) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
12) Wash the membrane with PBS-T (5 minutes x 3 times).
13) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
14) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

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