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AM26678AF-N Monoclonal Antibody to HA Epitope Tag (YPYDVPDYA) - Azide Free

Alternate names: HA Tag, HA-Tag, Hemagglutinin Tag

Quantity: 0.1 ml

Concentration: 0.2 mg/ml

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.

Host / Isotype: Mouse / IgG1

Clone: 5D8

Immunogen: Carrier protein (CP) conjugated peptide CP-YPYDVPDYA

Format:
State: Liquid Ig fraction
Purification: Protein A agarose
Buffer System: PBS containing 50% glycerol, pH 7.2, without preservatives

Applications: Western blot: 1 µg/mL for a chemiluminescence detection system.
Immunoprecipitation: 1 µg/Sample.
For details see protocols bellow.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with N-terminal and C-terminal HA-Tag.

Add. Information: This product was originally produced by MBL International.

Storage: Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Protocols:

SDS-PAGE & Western Blotting

1) Wash cells (approximately 5 x 10e6 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/cm2 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) Boil the samples for 2 minutes and centrifuge. Load 10µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
5) 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 4oC.

6) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
8) Incubate the membrane with 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) 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.
11) Expose the membrane onto an X-ray film under usual settings. The conditions for exposure and development may vary.

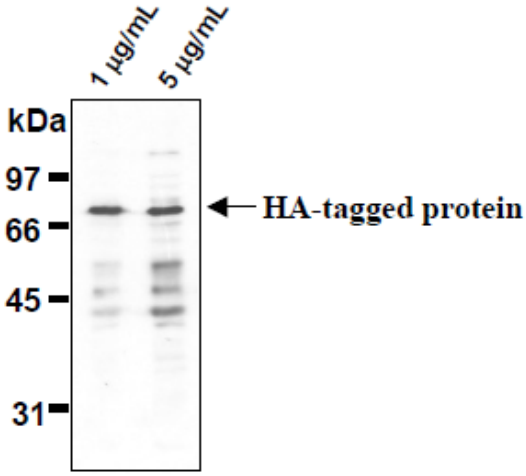
Immunoprecipitation

1) Wash cells (approximately 1 x 10e7 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 4oC 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 4oC 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 4oC.
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 4oC.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
7) Load 20 µL of the sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
8) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 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.
9) 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 4oC.
10) Incubate the membrane with 1:1,000 of anti-HA-Tag polyclonal antibody diluted with PBS, pH 7.2 containing 1% skimmed for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
12) Incubate the membrane with 1:10,000 HRP-conjugated anti-rabbit IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
13) Wash the membrane with PBS-T (5 minutes x 3 times).
14) Wipe excess buffer off the membrane, and incubate the membrane with an appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the

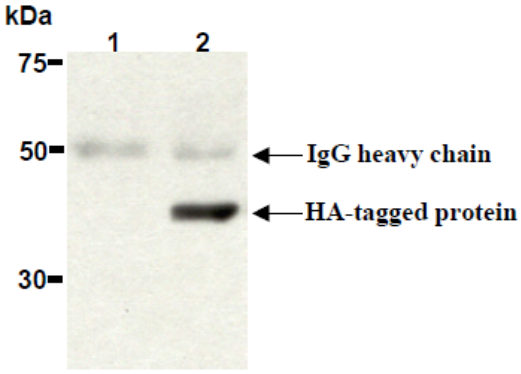
membrane by dabbing with a paper towel, and seal it in plastic wrap.
15) Expose the membrane onto an X-ray film in a dark room for 5 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

Pictures:

Western blot analysis of HA-tagged protein in transfectant using AM26678AF-N.



Immunoprecipitation of HA tagged transfectant with mouse IgG1 isotype control (1) or AM26678AF-N (2). After immunoprecipitatedwith the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-HA-tag polyclonal antibody.



Western blot analysis of various HA-tagged protein samples using AM26678AF-N.