

POLYCLONAL ANTIBODY

Anti-HA-tag pAb

Code No.
561

Quantity
100 μ L

Form
Rabbit IgG

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 rabbit serum using protein A agarose. The rabbit was immunized with KLH conjugated synthetic peptide, KLH-YPYDVPDYA (HA-tag).

FORMULATION: 100 μ L 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 HA-tag on Western blotting.

APPLICATIONS:

Western blotting; 1:1,000

Immunoprecipitation; 1 μ L

Immunohistochemistry; Not tested

Immunocytochemistry; 1:200

Flow cytometry; Not tested

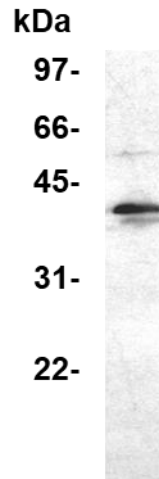
Detailed procedure is provided in the following
PROTOCOLS.

INTENDED USE:

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

REFERENCES:

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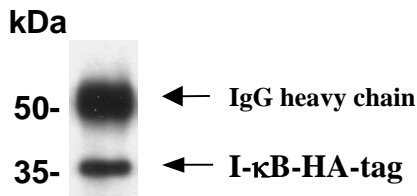
Western blotting analysis of HA-tagged I- κ B protein expression in 293T using 561.

PROTOCOLS:

SDS-PAGE & Western blotting

- 1) Mix the sample with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for 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% 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) for 1 hour at room temperature, or overnight at 4 $^{\circ}\text{C}$.
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (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 the 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.
- 8) Wash the membrane with PBS-T (5 minutes x 6 times).

- 9) 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.
- 10) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of HA-tagged I-κB protein from transfectant with 561. After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with 561.

Immunoprecipitation

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) 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 primary antibody as suggested in the **APPLICATIONS** into 200 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 μL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
- 5) Resuspend the beads with cold Lysis buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
- 7) Repeat steps 5)-6) 3-5 times
- 8) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 μL/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting.**)

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide.
- 2) Fix the cells by immersing the slide in acetone for 10 minutes on ice.
- 3) Air dry the slides.
- 4) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition are

recommended if necessary.)

- 5) Prepare a wash container such as a 500 mL beaker with a stirrer. Then wash the 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 wash once more.
- 6) Add 30 μL of 1:40 Anti-IgG (Rabbit) pAb-FITC (Beckman Coulter; code no. IM0833) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 4) Wash the slide in a plenty of PBS as in the step 5).
- 5) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 6) Promptly add mounting medium onto the slide, then put a cover slip on it.

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