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MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-T7-tag pAb

Code No.	Quantity	Form
PM022	100 µL	Affinity Purified

BACKGROUND: Epitope tagging is a powerful and versatile strategy for detecting and purifying proteins expressed by cloned genes. Short sequences encoding the epitope tag are cloned in-frame with target DNA to produce fusion proteins containing the epitope tag peptide. Due to their small size, epitope tags do not affect the tagged protein's biochemical properties. Anti-epitope tag antibodies can serve as universal purification or detection reagents for any tag-containing protein. Anti-T7-tag antibody is directed against the 11 amino acid of *gene 10* leader peptide (MASMTGGQQMG) expressed by many translation vectors. Because the peptide is the natural amino terminal end of the T7 major capsid protein, the antibody also recognizes T7 bacteriophage. Anti-T7-tag antibody is a useful reagent to easily identify, detect, or purify of T7-tag fusion proteins from cell lysates.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with carrier protein (*CP*) conjugated synthetic peptide, *CP*-MASMTGGQQMG.

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with T7-tag on Western blotting and Immunoprecipitation.

APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; 5 µL/sample
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested
Chromatin Immunoprecipitation; Not tested*
*It is reported that this polyclonal antibody can be used in Chromatin Immunoprecipitation in the following

Ichiyama, K., *et al.*, *J. Biol. Chem.* **283**, 17003-17008 (2008).

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

INTENDED USE:
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REFERENCES:
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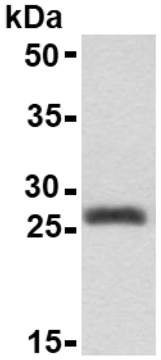


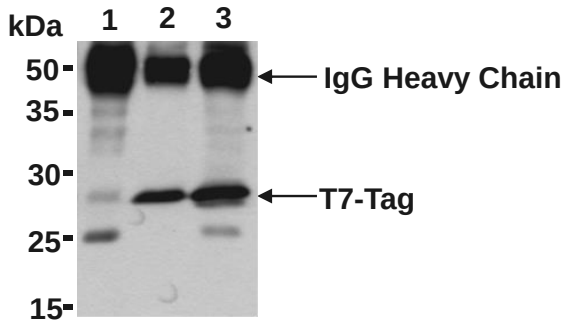
Western blotting analysis of T7-tagged protein using PM022

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

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 µL 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/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture'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°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 condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
7) Incubate the membrane with the 1:10,000 Anti-IgG (H+L chain) (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).
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 3 minutes. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of T7-tagged protein with normal from pET28a with normal rabbit IgG (1 mg) (1) or PM022 (1 mg) (2) or PM022 (5 mg) (3).
After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM022.

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 the antibody at the amount as suggested in the **APPLICATIONS** to the 200 µL of *E. coli* lysate. 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 Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
6) Resuspend the beads with cold Lysis buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
8) Repeat steps 6)-7) 3-5 times
9) 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**.)

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