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MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-T7-tag pAb-Agarose

Code No.	Quantity
PM022-8	Gel: 200 µL

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. The T7-tag antibody is directed against the 11 amino acid of *gene 10* leader peptide expressed by many translation vectors (MASMTGGQQMG). Because the peptide is the natural amino terminal end of the T7 major capsid protein, the antibody also recognizes T7 bacteriophage. Anti-T7-tag antibodies are useful reagents to easily identify, detect, or purify 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: 340 µg of anti-T7-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 T7-tag peptide sequence (MASMTGGQQMG) 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

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REFERENCE:

1) Sekiya, T., *et al.*, *Nat. Commun.* **2**, 269 (2011)

This antibody is used in this reference.

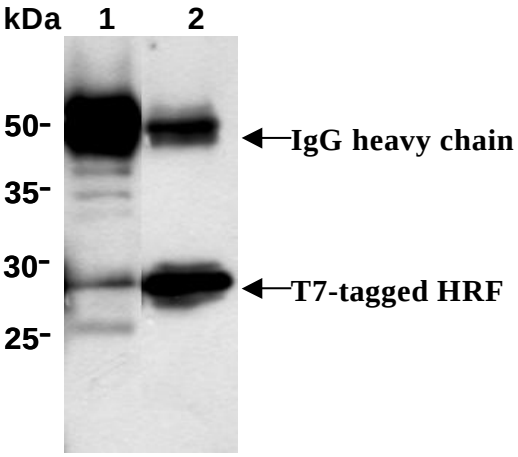
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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% methanol). 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 µg/mL of anti-T7-tag polyclonal antibody (MBL; code no. PM022) diluted with PBS, pH 7.2 containing 1% skimmed for 1 hour at room temperature. (The concentration of antibody to be used will depend on condition.)
10) Wash the membrane with PBS (5 minutes x 3).
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 (5 minutes x 3).

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.



Immunoprecipitation of T7-Tag from BL21/pET28a-HRF E. coli lysate with rabbit IgG (1) and PM022-8 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM022.*

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