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

POLYCLONAL ANTIBODY

Anti-S-tag pAb

Code No.	Quantity	Form
PM021	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. S-tag is an epitope tag composed of a 15 residues peptide, KETAAAKFERQHMDS, derived from the pancreatic ribonuclease A. S-tag can combine with S-protein to form functional RNase S, allowing detection and quantification of S-tag fusion proteins by enzymatic assays. However, antibody against the S-tag sequence provides a more convenient and flexible method to easily identify, detect, or purify S-tag containing fusion proteins.

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

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.

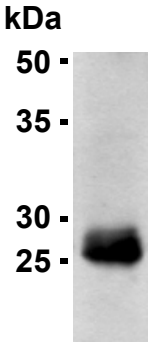
REACTIVITY: This antibody reacts with S-tagged protein on Western blotting and Immunoprecipitation.

APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; 5 µL/sample
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedures are provided in **PROTOCOLS**.

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

REFERENCE:
1) Kadooka, C., *et al., Appl. Environ. Microbiol.* **85**, e03136-18 (2019) [WB]
2) Zhao, J., *et al., Jundishapur J. Microbiol.* **11**, e68982 (2018) [WB]
3) O'Rourke, T. W. and Reines, D., *PLoS One* **11**, e0150865 (2016) [WB]
4) O'Rourke, T. W., *et al., Prion* **9**, 34-47 (2015) [WB]
5) Ogawa, D., *et al.*, *Plant Physiol. Biochem.* **61**, 54-60 (2012) [WB]



Western blot analysis of S-tagged HRF recombinant protein using PM021.

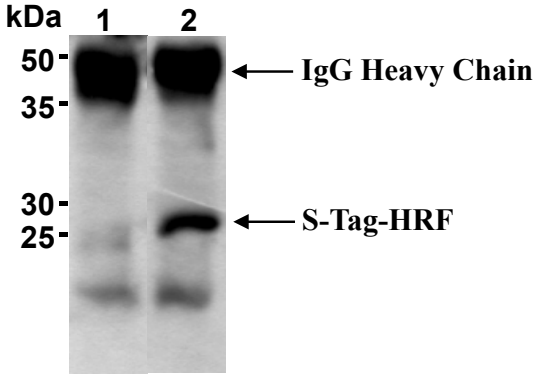
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 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/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.
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 for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (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 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 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.
10) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.



Immunoprecipitation of S-tagged HRF recombinant protein with normal rabbit IgG (1) or PM021 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM021.*

Immunoprecipitation

1) Add the antibody at the amount as suggested in **APPLICATIONS** to 200 µL of *E. coli* lysate and add 200 µL of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40]. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
2) Add 20 µL of 50% protein A agarose beads resuspended in the IP buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
3) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
4) Resuspend the agarose with cold Lysis buffer.
5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
6) Repeat steps 3)-5) 2-4 times.
7) 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 SDS-PAGE analysis.
   (See **SDS-PAGE & Western blotting**.)

For more information, please visit our website at https://ruo.mbl.co.jp/.