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My select sampler set

Anti-V5-tag pAb

Code No.	Quantity	Form
PM003MS	20 µL	Affinity Purified

BACKGROUND: The V5 tag epitope (GKPIPNPLLGLDST) is derived from P and V proteins of the paramyxovirus SV5. Expression vectors containing a protein and a tag peptide are commonly used. The V5-tagged protein expression system is preferably used in various laboratories. This specific antibody for the V5 tag epitope is a useful tool for monitoring of the V5-tagged protein.

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

FORMULATION: 20 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 recognizes V5-tag on Western blotting and Immunoprecipitation.

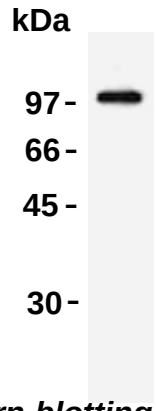
APPLICATIONS:
Western blotting; 1:2,000
Immunoprecipitation; 5 µL
Immunohistochemistry; Not tested
Immunofluorescence; Not tested*
Immunocytochemistry; Not tested
Flow cytometry; Not tested
Chromatin Immunoprecipitation; Not tested*
*It is reported that this antibody can be used in Immunofluorescence[1), 3), 8)] and Chromatin Immunoprecipitation[4), 7)].

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

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

REFERENCES:
1) Chen, X., *et al.*, *Protein Cell* **5**, 912-927 (2014) [WB, IF]
2) Sugiyama, T., *et al.*, *Nucleic Acids Res.* **41**, 6674-6686 (2013) [IP]
3) Maekawa, T., *et al.*, *Mol. Neurodegener.* **7**, 15 (2012) [WB, IF]
4) Benoki, S., *et al.*, *Arch. Biochem. Biophys.* **517**, 123-130 (2012) [ChIP]
5) Joo, J. Y., *et al.*, *Biochem. Biophys. Res. Commun*. **406**, 627-632 (2011) [IP]
6) Mimura, S., *et al.*, *J. Biol. Chem.* **285**, 9858-9867 (2010) [WB]
7) Yoshinari, K., *et al.*, *Biochem. Pharmacol.* **79**, 261-269 (2010) [ChIP]
8) Nadanaka, S., *et al., Mol. Cell Biol.* **27**, 1027-1043 (2007) [WB, IF]
9) Maeda, T., *et al., Blood* **105**, 2115-2123 (2005) [IP]
10) Gräler, M. H., Goetzl, E. J., *FASEB J.* **18**, 551-553 (2004) [WB]

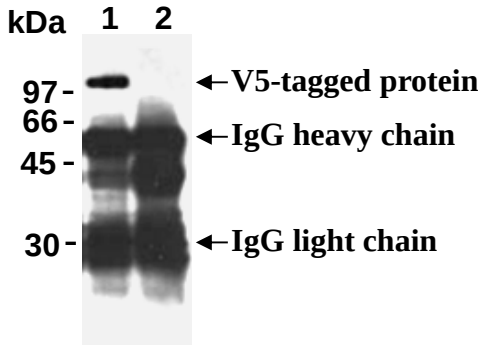
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Western blotting analysis of V5-tagged protein using PM003.

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 to be used 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 (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 30 seconds. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of V5-tagged protein

Lane 1: IP with Anti-V5-tag pAb (PM003)
Lane 2: IP with Normal Rabbit IgG

Immunoblotted with Anti-V5-tag pAb (PM003)

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 of as suggested in **APPLICATIONS** to the 100 µL of supernatant. 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 ice-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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