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## Anti-V5-tag pAb

**Code No.**  
PM003MS

**Quantity**  
20  $\mu$ L

**Form**  
Affinity Purified

**BACKGROUND:** The V5 tag epitope (GKPIPPLLGLDST) 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 column. The rabbit was immunized with carrier protein (CP) conjugated synthetic peptide, CP-GKPIPPLLGLDST.

**FORMULATION:** 20  $\mu$ L 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody recognizes V5-tag on Western blotting and Immunoprecipitation.

### APPLICATIONS:

Western blotting; 1:2,000

Immunoprecipitation; 5  $\mu$ L

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<sup>1), 3),</sup>

<sup>8)</sup> and Chromatin Immunoprecipitation<sup>4), 7)</sup>.

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

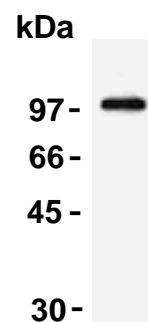
### INTENDED USE:

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]



**Western blotting analysis of V5-tagged protein using PM003.**

### PROTOCOLS:

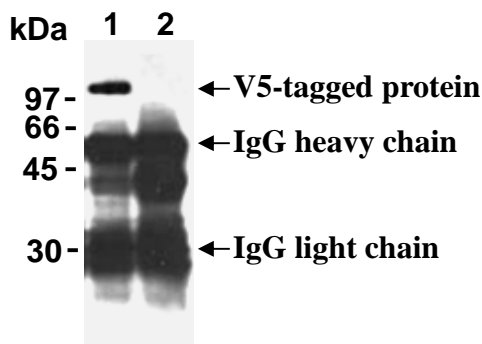
#### **SDS-PAGE & Western blotting**

- 1) Mix the sample with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10  $\mu$ 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/cm<sup>2</sup> 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.

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**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.**)