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

POLYCLONAL ANTIBODY

Anti-V5-tag pAb

Code No.	Quantity	Form
PM003	100 µL	Affinity Purified

BACKGROUND: Expression vectors containing a protein and a tag peptide are commonly used. V5-tag fusion protein expression system is preferably used in various laboratories. This specific antibody for V5-tag fusion protein is useful tools for monitoring of the fusion protein expression and affinity purification.

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: 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 recognizes recombinant V5-tag specifically on Western blotting.

APPLICATIONS:

Western blotting; 1:2,000 for chemiluminescence detection system
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)].

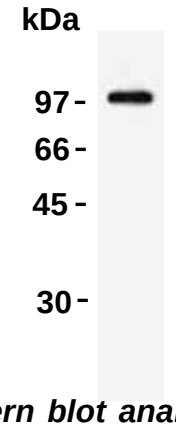
Detailed procedure is provided in the following **PROTOCOLS**.

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]



Western blot analysis of V5-tag expression in recombinant 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% MeOH). 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 PBS, pH 7.2 containing 1% skimmed milk as suggest 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 times).
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 times).

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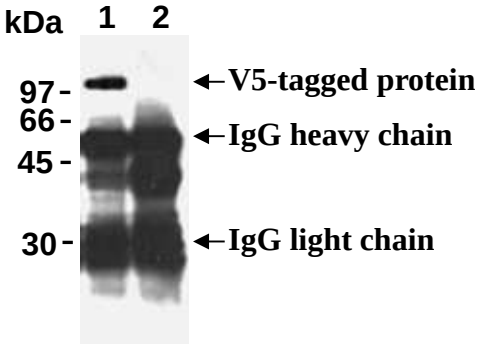
e-mail support@mbl.co.jp, TEL 052-238-1904

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-tag from recombinant with rabit IgG (1) or PM003 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with 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 suggest 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) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) 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**.)

RELATED PRODUCTS:

Antibodies

M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-DirecT (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M204-3	Anti-RFP mAb (1G9)
M204-7	Anti-RFP mAb-HRP-DirecT (1G9)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)
PM005	Anti-RFP pAb (polyclonal)
PM005-7	Anti-RFP pAb-HRP-DirecT (polyclonal)
M180-3	Anti-HA-tag mAb (TANA2) (200 µL)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-7	Anti-HA-tag pAb-HRP-DirecT (polyclonal)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-7	Anti-DDDDK-tag pAb-HRP-DirecT (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
M192-3	Anti-Myc-tag mAb (My3) (200 µL)
M047-3	Anti-Myc-tag mAb (PL14)
562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
D291-3	Anti-His-tag mAb (OGHis) (200 µL)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
PM032	Anti-His-tag pAb (polyclonal)
PM032-8	Anti-His-tag pAb-Agarose (polyclonal)
M167-3	Anti-V5-tag mAb (1H6)
PM003	Anti-V5-tag pAb (polyclonal)
PM003-7	Anti-V5-tag pAb-HRP-DirecT (polyclonal)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)
PM021	Anti-S-tag pAb (polyclonal)
PM070	Anti-E-tag pAb (polyclonal)
PM022	Anti-T7-tag pAb (polyclonal)
563	Anti-VSV-G-tag pAb (polyclonal)
M071-3	Anti-GST-tag mAb (3B2)
PM022	Anti-GST-tag pAb (polyclonal)
M095-3	Anti-Luciferase mAb (2D4)
PM016	Anti-Luciferase pAb (polyclonal)
PM047	Anti-Renilla Luciferase pAb (polyclonal)
M094-3	Anti-β-galactosidase mAb (5A3)
M203-3	Anti-β-galactosidase mAb (6F4)
PM049	Anti-β-galactosidase pAb (polyclonal)
M091-3	Anti-MBP (Maltose Binding Protein) mAb (1G12)
M013-3	Anti-Thioredoxin (Trx-tag) mAb (2C9)
PM015	Anti-CBD (Chitin Binding Domain) pAb (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)
M211-3	Anti-Strep-tag II mAb (4F1)
M214-3	Anti-mini-AID-tag mAb (1E4)

Protein Purification Kits

3320	HA-tagged Protein PURIFICATION KIT
3325	DDDDK-tagged Protein PURIFICATION KIT
3305	c-Myc-tagged Protein MILD PURIFICATION KIT Ver. 2
3310	His-tagged Protein PURIFICATION KIT
3317	V5-tagged Protein PURIFICATION KIT Ver.2
3318	V5-tagged Protein PURIFICATION GEL Ver.2
3315-205	V5-tag peptide
3341	V5-tagged Protein Magnetic PURIFICATION KIT

Other related antibodies and kits are also available.
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