

POLYCLONAL ANTIBODY

Anti-V5-tag pAb-Agarose

Code No.
PM003-8

Quantity
Gel: 200 μ L

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 a useful tool for monitoring of the fusion protein expression and affinity purification.

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: 400 μ g of anti-V5-tag polyclonal antibody covalently coupled to 200 μ L 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 μ L.

*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 V5-tag peptide sequence (GKPIPPLLGLDST) on Immunoprecipitation.

APPLICATIONS:

Western blotting; Not tested

Immunoprecipitation; 20 μ L of gel slurry

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow cytometry; Not tested

Detailed procedure is provided in the following
PROTOCOL.

INTENDED USE:

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

REFERENCES:

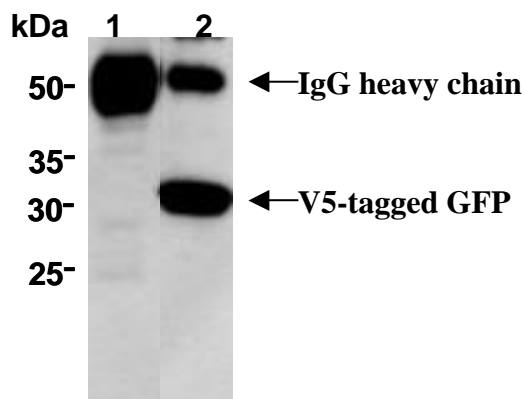
- 1) Benoki, S., *et al.*, *Arch. Biochem. Biophys.* **517**, 123-130 (2012)
- 2) Joo, J. Y., *et al.*, *Biochem. Biophys. Res. Commun.* **406**, 627-632 (2011)
- 3) Miura, S., *et al.*, *J. Biol. Chem.* **285**, 9858-9867 (2010)
- 4) Maeda, T., *et al.*, *Blood* **105**, 2115-2123 (2005)
- 5) Gräler, M. H., *et al.*, *FASEB J.* **18**, 551-553 (2004)

This antibody is used in these references.

PROTOCOL:

Immunoprecipitation

- 1) Add agarose as suggest in the **APPLICATIONS** into 1 μ g protein in 100 μ L of Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
- 2) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 3) Resuspend the agarose in 20 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 4) Load 20 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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.
- 6) 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.
- 7) Incubate the membrane with the 1:1,000 anti-V5-tag polyclonal antibody (MBL; code no. PM003) diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody to be used will depend on condition.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) 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.
- 10) Wash the membrane with PBS-T (5 minutes x 3 times).
- 11) 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.
- 12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of V5-Tag from V5 tagged GFP protein with rabbit IgG (1) and PM003-8 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM003.

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| 3310A | His-tagged Protein Purification Kit (Trial Kit) |
| 3310-205 | His-tag peptide (10mg) |
| 3311 | His-tagged Protein Purification Gel with Elution Peptide (1 mL gel, 10 mg peptide) |
| 3312 | His-tagged Protein Purification Gel with Elution Peptide (5 mL gel, 50 mg peptide) |
| 3315 | V5-tagged Protein Purification Kit |
| 3315A | V5-tagged Protein Purification Kit (Trial Kit) |
| 3320 | HA-tagged Protein Purification Kit |
| 3320A | HA-tagged Protein Purification Kit (Trial Kit) |
| 3320-205 | HA-tag peptide (10 mg) |
| 3321 | HA-tagged Protein Purification Gel (1 mL) |
| 3325 | DDDDK-tagged Protein Purification Kit |
| 3325A | DDDDK-tagged Protein Purification Kit (Trial Kit) |
| 3325-205 | DDDDK-tag peptide (5 mg) |
| 3326 | DDDDK-tagged Protein Purification Gel with Elution Peptide (1 mL gel, 5 mg peptide) |
| 3327 | DDDDK-tagged Protein Purification Gel with Elution Peptide (5 mL gel, 25 mg peptide) |
| 3328 | DDDDK-tagged Protein Purification Gel (5 mL gel) |
| 3329 | DDDDK-tagged Protein Purification Gel (25 mL gel) |

RELATED PRODUCTS:

Antibodies

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|---------|-------------------------------------|
| PM003 | anti-V5-tag (polyclonal) |
| M167-3 | anti-V5-tag (1H6) |
| PM003-7 | anti-V5-tag HRP-Direct (polyclonal) |
| PM003-8 | anti-V5-tag-agarose (polyclonal) |
| D291-8 | anti-His-tag-agarose (OGHis) |
| PM032-8 | anti-His-tag-agarose (polyclonal) |
| M047-8 | anti-Myc-tag-agarose (PL14) |
| PM020-8 | anti-DDDDK-tag-agarose (polyclonal) |
| D153-8 | anti-GFP-agarose (RQ2) |
| M165-8 | anti-RFP-agarose (3G5) |
| PM021-8 | anti-S-tag-agarose (polyclonal) |
| PM022-8 | anti-T7-tag-agarose (polyclonal) |
| 561-8 | anti-HA-tag-agarose (polyclonal) |
| 563-8 | anti-VSV-G-tag-agarose (polyclonal) |

Smart-IP series

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| 3190 | Magnetic Rack |
| M167-9 | anti-V5-tag-Magnetic beads (1H6) |
| M185-9 | anti-DDDDK-tag-Magnetic beads (FLA-1) |
| D291-9 | anti-His-tag-Magnetic beads (OGHis) |
| D153-9 | anti-GFP-Magnetic beads (RQ2) |
| M165-9 | anti-RFP-Magnetic beads (3G5) |
| M132-9 | anti-HA-tag-Magnetic beads (5D8) |
| M180-9 | anti-HA-tag-Magnetic beads (TANA2) |
| M047-9 | anti-Myc-tag-Magnetic beads (PL14) |
| D058-9 | anti-Multi Ubiquitin-Magnetic beads (FK2) |

Protein Purification Kit

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| 3305 | c-Myc-tagged Protein Mild Purification Kit |
| 3305A | c-Myc-tagged Protein Mild Purification Kit (Trial Kit) |
| 3306 | c-Myc-tagged Protein Mild Purification Gel with Elution Peptide (1 mL gel, 1 mg peptide) |
| 3307 | c-Myc-tagged Protein Mild Purification Gel with Elution Peptide (5 mL gel, 5 mg peptide) |
| 3300-205 | c-Myc-tag peptide (5 mg) |
| 3310 | His-tagged Protein Purification Kit |