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

MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-V5-tag pAb-Agarose

Code No.	Quantity
PM003-8	Gel: 200 µL

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*-GKPIPNPLLGLDST.

FORMULATION: 400 µg of anti-V5-tag polyclonal antibody covalently coupled to 200 µL 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 µL.

*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 (GKPIPNPLLGLDST) on Immunoprecipitation.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; 20 µL of gel slurry
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

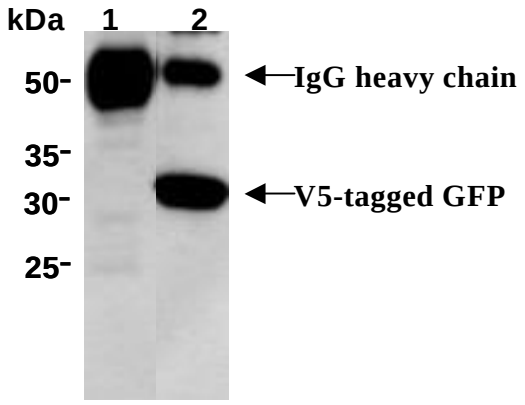
Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:

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

REFERENCES:

Please visit our website at https://ruo.mbl.co.jp/.



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.*

PROTOCOL:

Immunoprecipitation

1) Add primary antibody as suggested in the **APPLICATIONS** into 1 µg protein in 100 µL 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) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
3) Resuspend the beads with cold Lysis buffer.
4) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
5) Repeat steps 3)-4) 3-5 times
6) Resuspend the agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
7) Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
8) 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 manufacturer's manual for precise transfer procedure.
9) 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.
10) Incubate the membrane with 1:1,000 of Anti-V5-tag pAb (MBL, code no. PM003) diluted with 1% skimmed milk

(in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody to be used will depend on the conditions.)

11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
12) Incubate the membrane with 1:10,000 of 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.
13) Wash the membrane with PBS-T (5 minutes x 3).
14) 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.
15) 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.

RELATED PRODUCTS:

Please visit our website at https://ruo.mbl.co.jp/.