

MONOCLONAL ANTIBODY

Anti-V5-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M167-3	1H6	Mouse IgG2a κ	100 μ L	1 mg/mL

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 hybridoma (clone 1H6) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with carrier protein (CP) conjugated synthetic peptide, CP-GKPIPPLLGLDST.

FORMULATION: 100 μ g IgG in 100 μ L volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C .

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

APPLICATIONS:

Western blotting; 1 μ g/mL

Immunoprecipitation; 5 μ g/sample

Immunocytochemistry; 5 μ g/mL

Immunohistochemistry; Not tested

Flow cytometry; Not tested

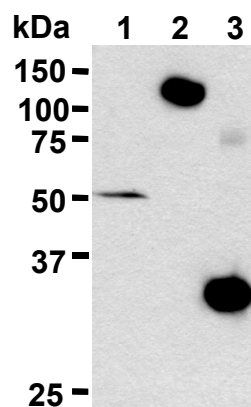
Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:

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

REFERENCES:

- 1) Jia, N., *et al.*, *Nat. Protoc.* **10**, 12-24 (2015) [IC]
- 2) Murthy, A., *et al.*, *Nature* **506**, 456-462 (2014) [WB]
- 3) Kashiyama, K., *et al.*, *Am. J. Hum. Genet.* **92**, 807-819 (2013) [WB]



Western blot analysis of V5-tag expression in V5 tagged proteins using M167-3.

Lane1: V5 tagged Foxp3 transfectant cell lysate

Lane2: V5 tagged TPO protein

Lane3: V5 tagged GFP protein

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

PROTOCOLS:

SDS-PAGE & Western Blotting

- 1) Wash cells at a concentration of 1×10^7 3 times with PBS and resuspend with 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 20 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 3) 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 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 suggested in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1%

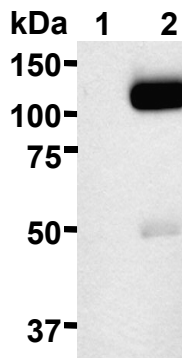
skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.

- 8) Wash the membrane with PBS-T (5 minutes x 3 times).
- 9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
- 10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 11) Expose the membrane onto an X-ray film in a dark room for 5 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

Immunoprecipitation

- 1) Wash the cells 3 times with PBS and resuspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) 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 primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis.

(See **SDS-PAGE & Western blotting.**)

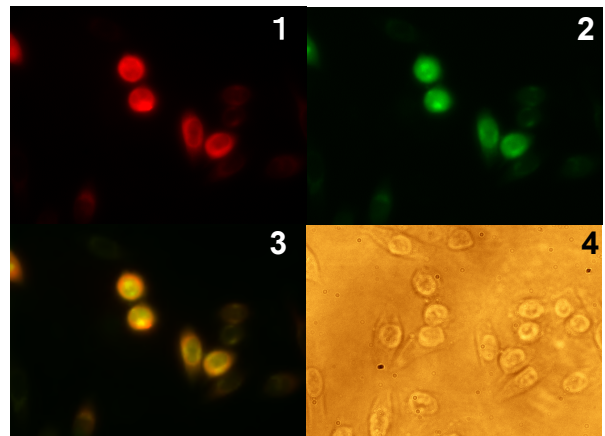


Immunoprecipitation of V5 tagged protein with Mouse IgG2a (1) or M167-3 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-V5-Tag polyclonal (MBL; code no. PM003).

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1×10^4 cells for one slide, then incubate in a CO₂ incubator for one night.)
- 2) Wash the cells 3 times with PBS.

- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 10 minutes at room temperature.
- 4) Wash the glass slide 2 times with PBS.
- 5) Immerse the slide in PBS containing 0.2% TritonX-100 for 20 minutes at room temperature.
- 6) Wash the glass slide 2 times with PBS.
- 7) Add the primary antibody diluted with PBS containing 2% FCS as suggested in the **APPLICATIONS** onto the cells and incubate for 60 minutes at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
- 8) Wash the glass slide 2 times with PBS.
- 9) Add 200 µL of 1:500 Alexa594 conjugated anti-mouse IgG (Invitrogen; code no. A21237) diluted with PBS containing 2% FCS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 10) Wash the glass slide 2 times with PBS-T.
- 11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunocytochemical detection of V5-tag in V5 tagged GFP transfected.

Panel 1: anti-V5-Tag (M167-3)

Panel 2: GFP own fluorescence

Panel 3: Merge

Panel 4: Transmission light

Microscope; Axiovert 200 (Carl Zeiss, Inc)(40 x, NA0.6)

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