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Anti-V5-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M167-3MS	1H6	Mouse IgG2a κ	20 µL	1 mg/mL

BACKGROUND: The V5 tag epitope (GKPIPNPLLGLDST) 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*-GKPIPNPLLGLDST.

FORMULATION: 20 µg IgG in 20 µL 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 µg/mL
Immunoprecipitation; 5 µg/sample
Immunocytochemistry; 5 µg/mL
Immunohistochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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REFERENCES:

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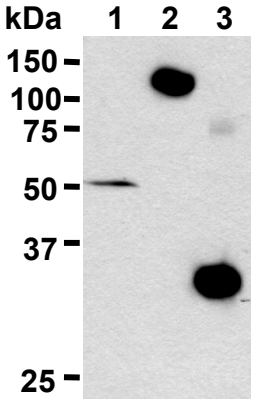


Western blotting analysis of V5-tagged proteins using M167-3.
Lane 1: V5-tagged Foxp3 in transfected cell lysate
Lane 2: V5-tagged TPO protein
Lane 3: V5-tagged GFP protein

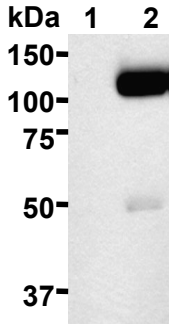
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash cells at a concentration of 1 x 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 µL 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/cm^2 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 will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
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).

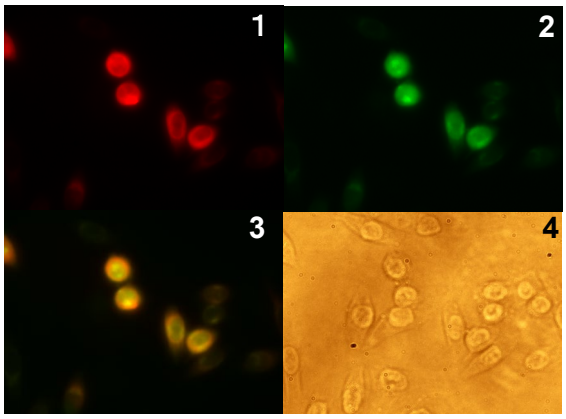
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 5 minutes. Develop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



Immunoprecipitation of V5-tagged protein.
Lane 1: IP with Mouse IgG2a (isotype control) (code no. M076-3)
Lane 2: IP with Anti-V5-tag mAb (code no. M167-3)
Immunoblotted with Anti-V5-tag pAb (code no. PM003)

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 4ºC 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 4ºC. 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) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
5) Resuspend the beads with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
7) Repeat steps 5)-6) 3-5 times
8) 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**.)



Immunocytochemical detection of V5-tag in V5-tagged GFP transfectant.
Panel 1: Anti-V5-tag mAb (code no. M167-3)
Panel 2: GFP own fluorescence
Panel 3: Merge, 1 and 2
Panel 4: Phase-contrast image
Microscope; Axiovert 200 (Carl Zeiss, Inc)(40 x, NA0.6)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator overnight.)
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 twice with PBS.
5) Immerse the slide in PBS containing 0.2% Triton X-100 for 20 minutes at room temperature.
6) Wash the glass slide twice 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 twice with PBS.
9) Add 200 µL of 1:500 Alexa594 conjugated anti-mouse IgG (Thermo Fisher Scientific; code no. A-21237) 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 twice with PBS.
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.

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