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Not for use in diagnostic procedures.

MBL
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Anti-V5-tag mAb

CODE No.	M215-3
CLONALITY	Monoclonal
CLONE	OZA3
ISOTYPE	Mouse IgG2b κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Carrier protein conjugated synthetic peptide, GKPIPNPLLGLDST (V5-tag)
FORMULATION	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.

APPLICATIONS-CONFIRMED

Western blotting	1 µg/mL
Immunoprecipitation	2.5 µg/sample
Immunocytochemistry	1 µg/mL
Flow cytometory	0.5 µg/mL

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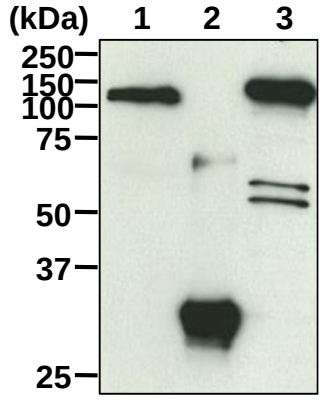
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 1 x 10^6 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3].
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3).
8) Incubate the membrane with the 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 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3).
10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



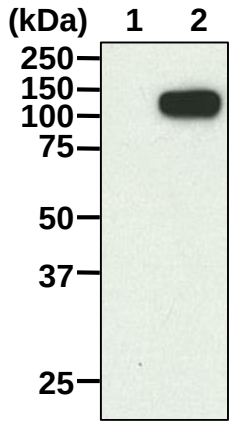
Western blotting analysis of V5-tagged proteins

Lane 1: V5-tagged TPO in insect cell culture sup (5 μL/lane)
Lane 2: V5-tagged GFP (25 ng/lane)
Lane 3: V5-tagged β-galactosidase/HEK293T

Immunoblotted with Anti-V5-tag mAb (M215-3)

Immunoprecipitation

1) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 300 µL of IP buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
2) Wash the beads 1 time with 1 mL of IP buffer.
3) Add 100 µL of culture supernatant and 200 µL of IP buffer, then incubate with gentle agitation for 1 hr. at 4°C.
4) Wash the beads 4 times with 1 mL of IP buffer.
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 2 min. and centrifuge.
6) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
7) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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.
8) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
10) Incubate the membrane with 1:1,000 of Anti-V5-tag pAb-HRP-DirecT (MBL; code no. PM003-7) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
11) Wash the membrane with PBS-T (5 min. x 3).
12) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
13) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
14) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



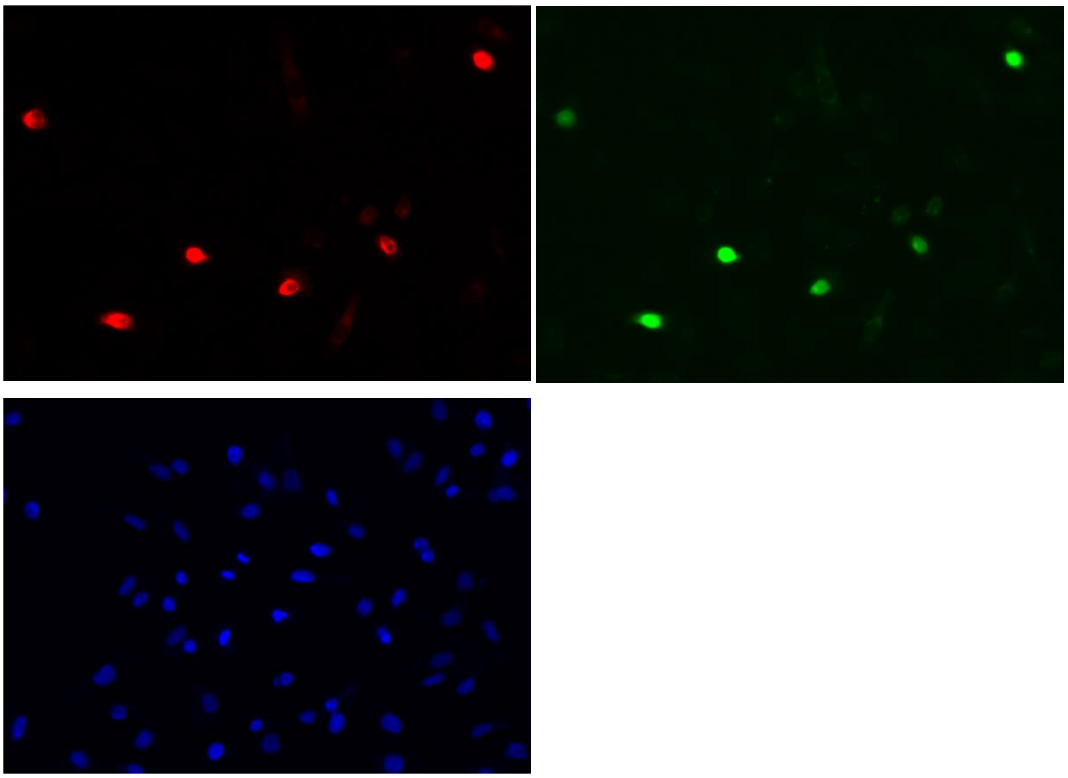
Immunoprecipitation of V5-tagged protein from insect cell culture supernatant

Sample: Insect cell culture sup. containing V5-tagged TPO
Lane 1: Mouse IgG2b (isotype control) (M077-3)
Lane 2: Anti-V5-tag mAb (M215-3)

Immunoblotted with Anti-V5-tag pAb-HRP-DirecT (PM003-7)

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide twice with PBS.
4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
5) Wash the slide twice with PBS.
6) Permeabilize the cells with 200 µL of 0.2% Triton X-100/PBS for 10 min. at room temperature.
7) Wash the slide twice with PBS.
8) Tip off PBS and add 200 µL of the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide twice with PBS.
10) Add 100 µL of 1:500 Alexa Fluor® 594 Goat Anti-mouse IgG (Invitrogen; code no. A11005) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide twice with PBS.
12) Wipe excess liquid from the slide but take care not to touch the cells. Never leave the cells to dry.
13) Counterstain with DAPI for 5 min. at room temperature.
14) Wash the slide twice with PBS.
15) Promptly add mounting medium onto the slide, then put a cover slip on it.

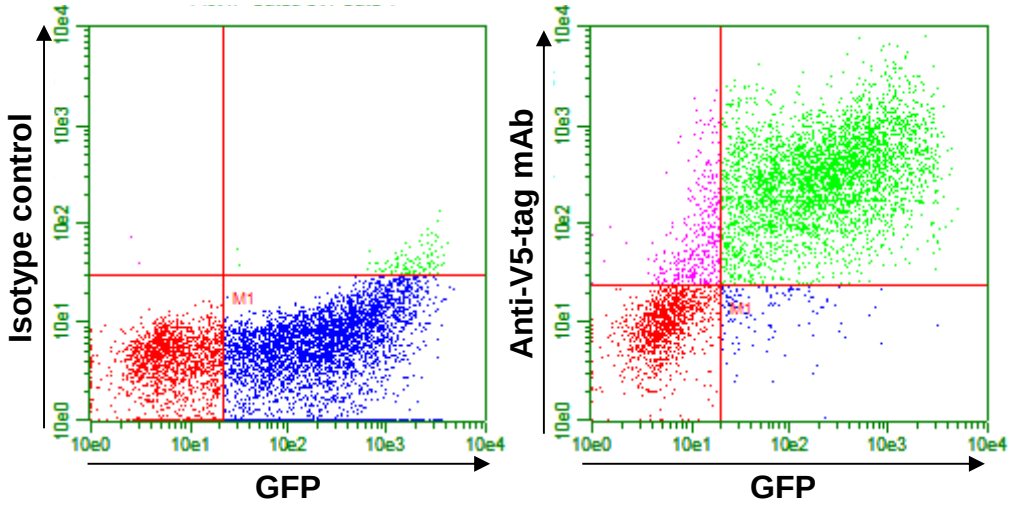


Immunocytochemical detection of V5-tagged GFP in HeLa transfectant

Red: Anti-V5-tag mAb (M215-3)
Green: V5-tagged GFP own fluorescence
Blue: DAPI

Flow cytometric analysis

1) Wash 5 x 10^5 cells 3 times with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
2) Add 100 µL of 4% paraformaldehyde (PFA)/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
3) Wash the cells 1 time with 1 mL of the washing buffer.
4) Add 100 µL of 0.2% Triton X-100/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
5) Wash the cells 1 time with 1 mL of the washing buffer.
6) Add 50 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
7) Wash the cells 1 time with 1 mL of the washing buffer.
8) Add PE-conjugated anti-mouse IgG antibody diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
9) Wash the cells 1 time with 1 mL of the washing buffer.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.



Flow cytometric detection of V5-tagged GFP in HEK293T transfectant

Left: Mouse IgG2b (isotype control) (M077-3)
Right: Anti-V5-tag mAb (M215-3)