

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

 **My select** sampler set

Anti-V5-tag mAb

CODE No.	M215-3MS
CLONALITY	Monoclonal
CLONE	OZA3
ISOTYPE	Mouse IgG2b κ
QUANTITY	20 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Carrier protein conjugated synthetic peptide, GKPIPPLLGLDST (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

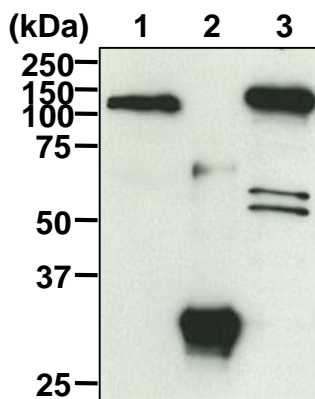
<u>Western blotting</u>	1 μ g/mL
<u>Immunoprecipitation</u>	2.5 μ g/sample
<u>Immunocytochemistry</u>	1 μ g/mL
<u>Flow cytometry</u>	0.5 μ g/mL

For more information, please visit our web site <https://ruo.mbl.co.jp/>.

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

SDS-PAGE & Western blotting

- 1) Wash 1×10^6 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 μ L 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/cm² 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.



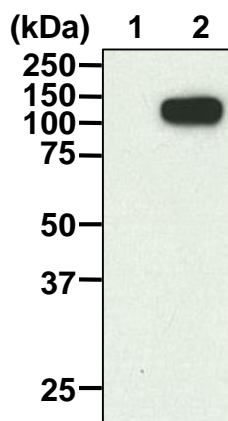
Western blotting analysis of V5-tagged proteins

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

Immunoblotted with Anti-V5-tag mAb (MBL; code no. M215-3)

Immunoprecipitation

- 1) Mix 20 μ L of 50% protein A agarose beads slurry resuspended in 300 μ L 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 once with 1 mL of IP buffer.
- 3) Add 100 μ L of culture supernatant and 200 μ L 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 μ L of Laemmli's sample buffer, boil for 2 min. and centrifuge.
- 6) Load 10 μ L 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/cm² 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

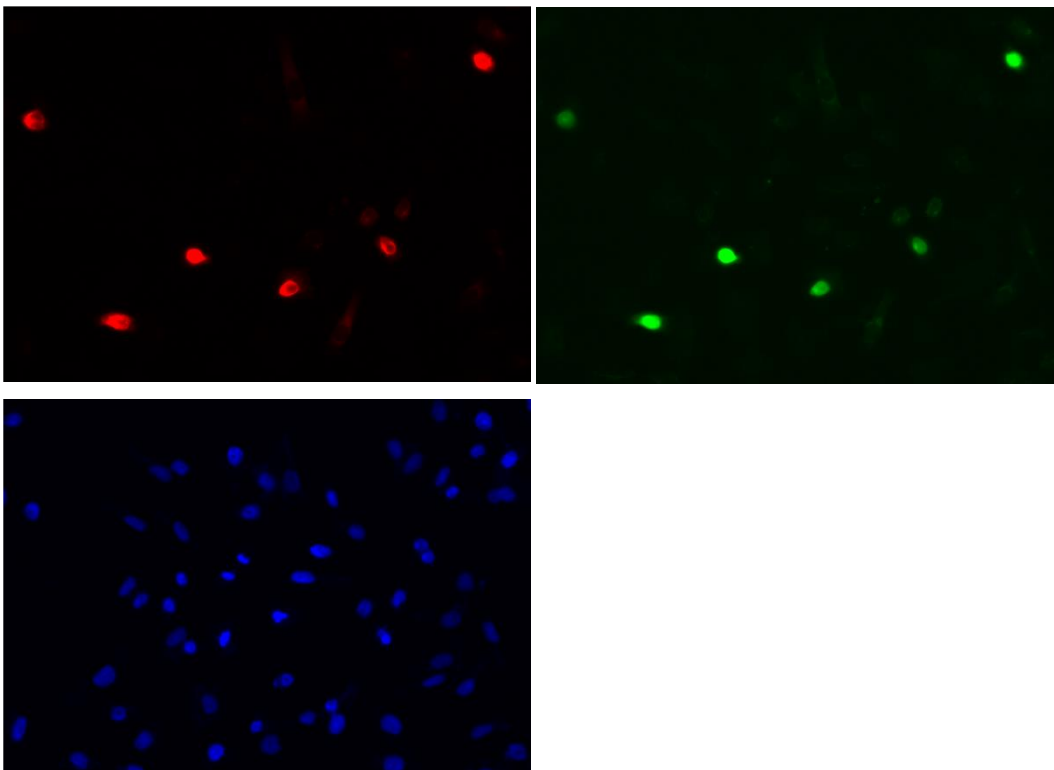
Lane 1: Mouse IgG2b (isotype control) (MBL; code no. M077-3)

Lane 2: Anti-V5-tag mAb (MBL; code no. M215-3)

Immunoblotted with Anti-V5-tag pAb-HRP-Direct (MBL; code no. PM003-7)

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ incubator overnight.
- 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 (Thermo Fisher Scientific; code no. A-11005) 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

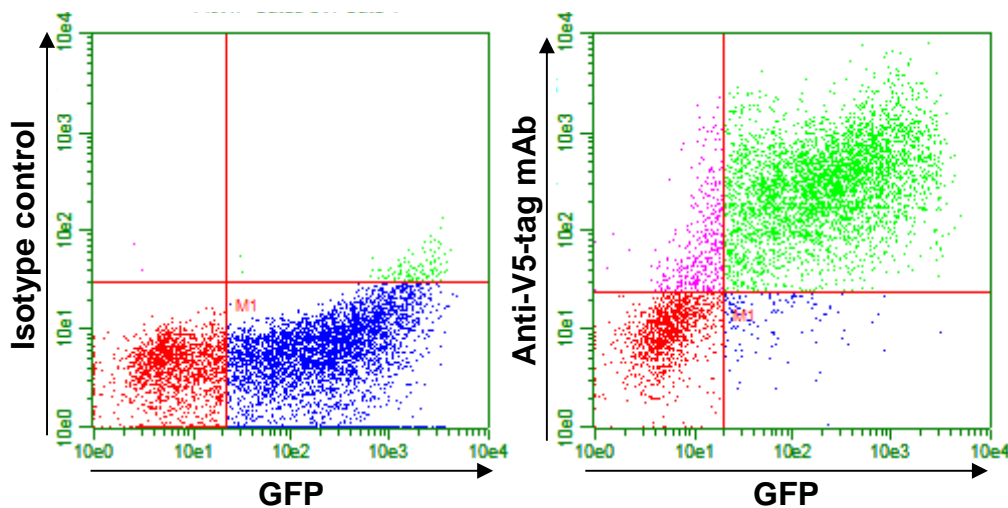
Red: Anti-V5-tag mAb (MBL; code no. M215-3)

Green: V5-tagged GFP own fluorescence

Blue: DAPI

Flow cytometric analysis

- 1) Wash 5×10^5 cells 3 times with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Add 100 μ L 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 once with 1 mL of the washing buffer.
- 4) Add 100 μ L 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 once with 1 mL of the washing buffer.
- 6) Add 50 μ L 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 once 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 once with 1 mL of the washing buffer.
- 10) Resuspend the cells with 500 μ L 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) (MBL; code no. M077-3)

Right: Anti-V5-tag mAb (MBL; code no. M215-3)