

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



## Anti-V5-tag mAb

<b>CODE No.</b>	M215-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	OZA3
<b>ISOTYPE</b>	Mouse IgG2b $\kappa$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	Carrier protein conjugated synthetic peptide, GKPIPPLLGLDST (V5-tag)
<b>FORMURATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	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 $\mu$ g/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	2.5 $\mu$ g/sample
<u>Immunocytochemistry</u>	1 $\mu$ g/mL
<u>Flow cytometry</u>	0.5 $\mu$ g/mL

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



## RELATED PRODUCTS

### Antibodies

M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-6	Anti-GFP mAb-Biotin (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-Direct (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
M204-3	Anti-RFP mAb (1G9)
M204-7	Anti-RFP mAb-HRP-Direct (1G9)
PM005	Anti-RFP pAb (polyclonal)
PM005-7	Anti-RFP pAb-HRP-Direct (polyclonal)
M180-3	Anti-HA-tag mAb (TANA2) (200 $\mu$ L)
M180-6	Anti-HA-tag mAb-Biotin (TANA2)
M180-7	Anti-HA-tag mAb-HRP-Direct (TANA2)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-7	Anti-HA-tag pAb-HRP-Direct (polyclonal)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
M185-7	Anti-DDDDK-tag mAb-HRP-Direct (FLA-1)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-7	Anti-DDDDK-tag pAb-HRP-Direct (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
M192-3	Anti-Myc-tag mAb (My3) (200 $\mu$ L)
M192-6	Anti-Myc-tag mAb-Biotin (My3)
M047-3	Anti-Myc-tag mAb (PL14)
M047-7	Anti-Myc-tag mAb-HRP-Direct (PL14)
M047-8	Anti-Myc-tag mAb-Agarose (PL14)
562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
D291-3	Anti-His-tag mAb (OGHis) (200 $\mu$ L)
D291-6	Anti-His-tag mAb-Biotin (OGHis)
D291-7	Anti-His-tag mAb-HRP-Direct (OGHis)
D291-8	Anti-His-tag mAb-Agarose (OGHis)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
PM032	Anti-His-tag pAb (polyclonal)
PM032-8	Anti-His-tag pAb-Agarose (polyclonal)
M167-3	Anti-V5-tag mAb (1H6)
M215-3	Anti-V5-tag mAb (OZA3)
PM003	Anti-V5-tag pAb (polyclonal)
PM003-7	Anti-V5-tag pAb-HRP-Direct (polyclonal)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)
PM021	Anti-S-tag pAb (polyclonal)
PM070	Anti-E-tag pAb (polyclonal)
PM022	Anti-T7-tag pAb (polyclonal)
563	Anti-VSV-G-tag pAb (polyclonal)
M071-3	Anti-GST-tag mAb (3B2)
M209-3	Anti-GST-tag mAb (GT5)
PM013	Anti-GST-tag pAb (polyclonal)
PM013-7	Anti-GST-tag pAb-HRP-Direct (polyclonal)
M095-3	Anti-Luciferase mAb (2D4)
PM016	Anti-Luciferase pAb (polyclonal)
PM047	Anti-Renilla Luciferase pAb (polyclonal)
M094-3	Anti- $\beta$ -galactosidase mAb (5A3)

PM049	Anti- $\beta$ -galactosidase pAb (polyclonal)
M091-3	Anti-MBP (Maltose Binding Protein) mAb (1G12)
M013-3	Anti-Thioredoxin (Trx-tag) mAb (2C9)
PM015	Anti-CBD (Chitin Binding Domain) pAb (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)
M211-3	Anti-Strep-tag II mAb (4F1)
M214-3	Anti-mini-AID-tag mAb (1E4)
M214-7	Anti-mini-AID-tag mAb-HRP-Direct (1E4)

### Smart-IP series

3190	Magnetic Rack
M180-11	Anti-HA-tag mAb-Magnetic Beads (TANA2)
M132-11	Anti-HA-tag mAb-Magnetic Beads (5D8)
M185-11	Anti-DDDDK-tag mAb-Magnetic Beads (FLA-1)
M047-11	Anti-Myc-tag mAb-Magnetic Beads (PL14)
D291-11	Anti-His-tag mAb-Magnetic Beads (OGHis)
D153-11	Anti-GFP mAb-Magnetic Beads (RQ2)
M165-11	Anti-RFP mAb-Magnetic Beads (3G5)
M167-11	Anti-V5-tag mAb-Magnetic Beads (1H6)
M198-9	Anti-E-tag mAb-Magnetic beads (21D11)
D058-9	Anti-Multi Ubiquitin mAb-Magnetic beads (FK2)
M075-11	Mouse IgG1 (isotype control)-Magnetic Beads
M076-11	Mouse IgG2a (isotype control)-Magnetic Beads
M077-11	Mouse IgG2b (isotype control)-Magnetic Beads
M081-11	Rat IgG2a (isotype control)-Magnetic Beads
M180-10	Anti-HA-tag mAb-Magnetic Agarose (TANA2)
M132-10	Anti-HA-tag mAb-Magnetic Agarose (5D8)
M185-10	Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
D291-10	Anti-His-tag mAb-Magnetic Agarose (OGHis)
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)

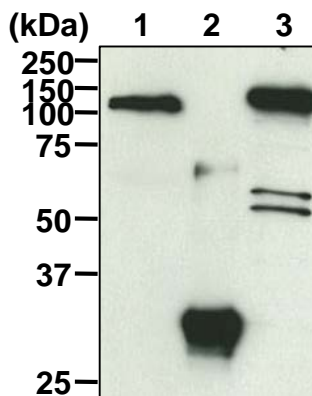
### Protein Purification Kits

3320	HA-tagged Protein PURIFICATION KIT
3321	HA-tagged Protein PURIFICATION GEL (1 mL)
3325	DDDDK-tagged Protein PURIFICATION KIT
3326	DDDDK-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
3325-205	DDDDK-tag peptide (1 mg x 5)
3326K	DDDDK-tagged Protein PURIFICATION CARTRIDGE (1 mL x 1)
3305	c-Myc-tagged Protein MILD PURIFICATION KIT
3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
3310	His-tagged Protein PURIFICATION KIT
3311	His-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3317	V5-tagged Protein PURIFICATION KIT Ver.2
3318	V5-tagged Protein PURIFICATION GEL Ver.2 (1 mL)
3315-205	V5-tag peptide (2 mg x 5)

Other related antibodies and kits are also available.  
Please visit our website at <http://ruo.mbl.co.jp/>

### **SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^6$  cells 3 times with PBS and suspend 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  $\mu$ 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/cm<sup>2</sup> for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). 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 times].
- 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 times).
- 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 times).
- 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 blot analysis of V5-tagged proteins***

Lane 1: V5-tagged TPO in insect cell culture sup (5  $\mu$ L/lane)

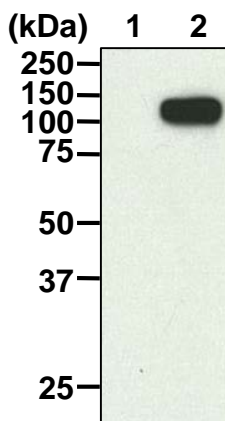
Lane 2: V5-tagged GFP (25 ng/lane)

Lane 3: V5-tagged  $\beta$ -galactosidase/HEK293T

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

### **Immunoprecipitation**

- 1) Mix 20  $\mu$ L of 50% protein A agarose beads slurry resuspended in 300  $\mu$ 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 1 time with 1 mL of IP buffer.
- 3) Add 100  $\mu$ L of culture supernatant and 200  $\mu$ 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  $\mu$ L of Laemmli's sample buffer, boil for 2 min. and centrifuge.
- 6) Load 10  $\mu$ 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/cm<sup>2</sup> for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). 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 times).
- 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 times).
- 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.



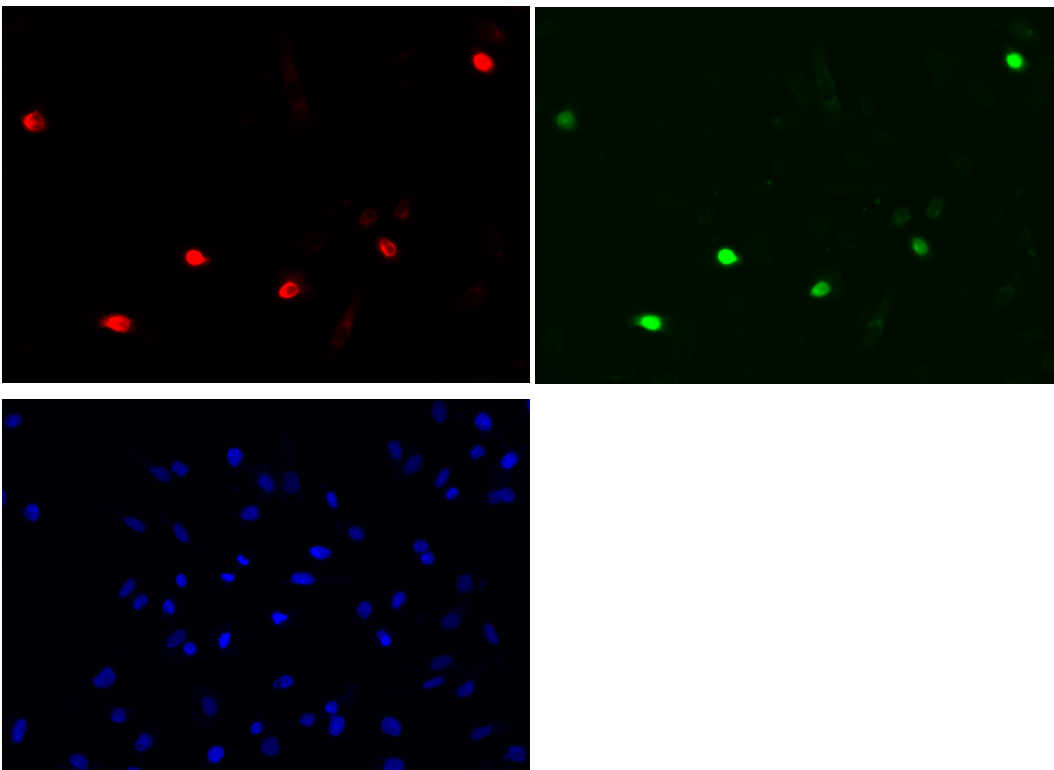
#### ***Immunoprecipitation of V5-tagged protein from insect cell culture supernatant***

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<sub>2</sub> incubator for one night.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide 2 times with PBS.
- 4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
- 5) Wash the slide 2 times 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 2 times 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 2 times with PBS.
- 10) Add 100 µL of 1:500 Alexa Fluor<sup>®</sup> 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 2 times 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 2 times with PBS.
- 15) Promptly add mounting medium onto the slide, then put a cover slip on it.

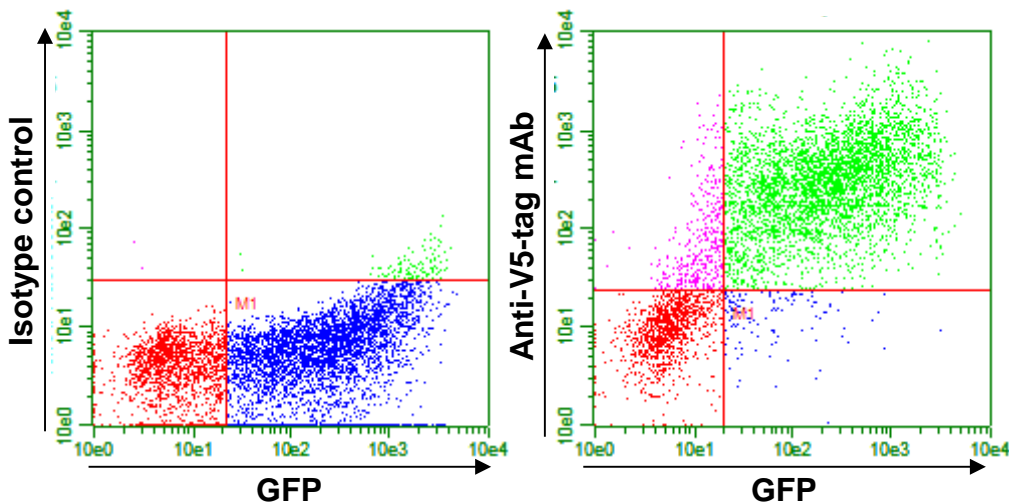


#### ***Immunocytochemical detection of V5-tagged GFP in HeLa transfectant***

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

### **Flow cytometric analysis**

- 1) Wash  $5 \times 10^5$  cells 3 times with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Add 100  $\mu$ 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 1 time with 1 mL of the washing buffer.
- 4) Add 100  $\mu$ 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 1 time with 1 mL of the washing buffer.
- 6) Add 50  $\mu$ 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 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  $\mu$ 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) (M077-3)  
Right: Anti-V5-tag mAb (M215-3)