

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



Anti-V5-tag mAb-Biotin

CODE No.	M215-6
CLONALITY	Monoclonal
CLONE	OZA3
ISOTYPE	Mouse IgG2b κ
QUANTITY	50 μ L
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Carrier protein conjugated synthetic peptide, GKPIPPLLGLDST (V5-tag)
FORMURATION	PBS (pH 7.2) containing 1% BSA and 0.1% ProClin 150
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at 4°C.
APPLICATIONS-CONFIRMED	
<u>Western blotting</u>	1:1,000 for chemiluminescence detection system
<u>Sandwich ELISA</u>	1:2,000 for chemiluminescence detection system

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 µL)
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 µL)
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 µL)
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)
M215-6 Anti-V5-tag mAb-Biotin (OZA3)
M215-7 Anti-V5-tag mAb-HRP-Direct (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-β-galactosidase mAb (5A3)
PM049 Anti-β-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)

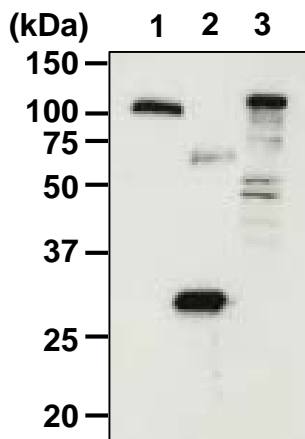
Protein Purification Kits

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)
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
3341 V5-tagged Protein Magnetic PURIFICATION KIT
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) Prepare samples described as below:
[Transfectant] 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.).
[Recombinant protein] Mix the samples with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 3 min. and centrifuge. Load 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% 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 1:20,000 of Streptavidin-Horseradish Peroxidase (GE Healthcare; code no. RPN4401) 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 3 min. Develop the film as usual. The condition for exposure and development may vary.



Western blot analysis of V5-tagged protein

Lane 1: V5-tagged TPO in insect cell culture sup. (10 μL /lane)

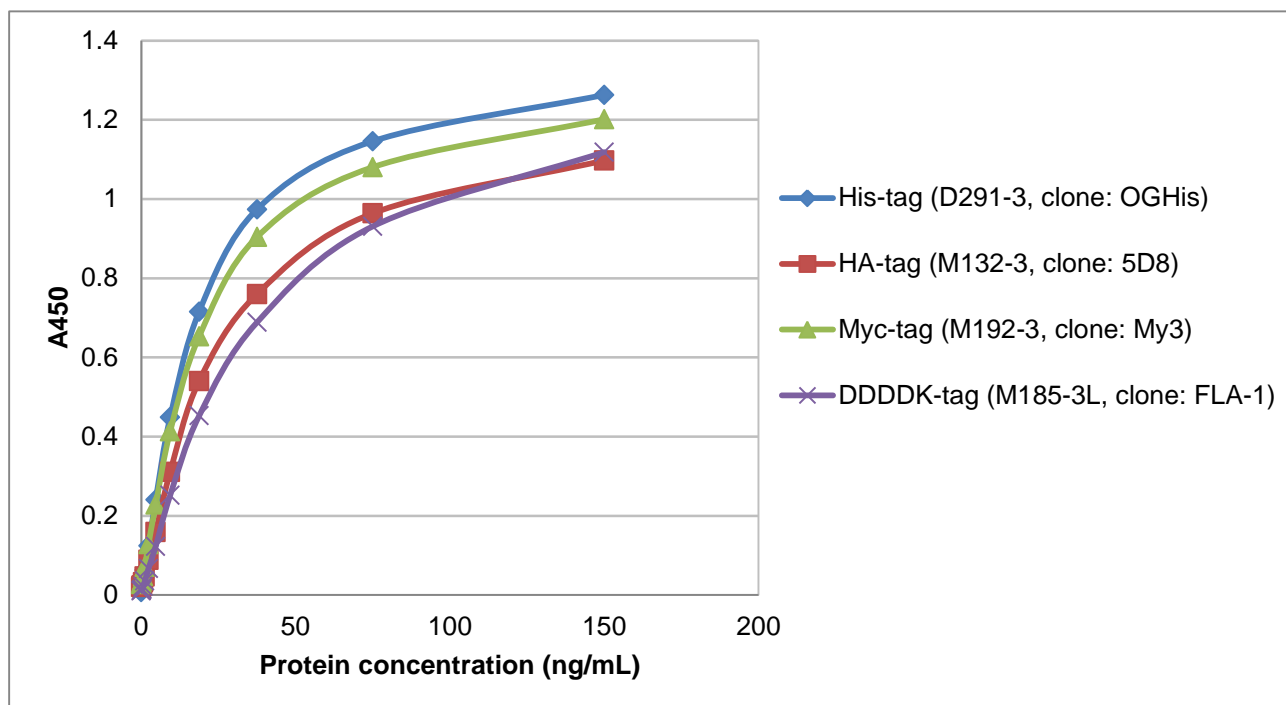
Lane 2: V5-tagged GFP (50 ng/lane)

Lane 3: V5-tagged β -galactosidase/HEK293T

Immunoblotted with Streptavidin-Horseradish Peroxidase

Sandwich ELISA

- 1) Add 100 μL /well of 5 $\mu\text{g}/\text{mL}$ capture antibody diluted 0.1 M Carbonate buffer (pH 9.5) to the 96-well microplate. Incubate for 1 hr. at room temperature.
- 2) Wash the plate with PBS (1 time).
- 3) Add 200 μL /well of Blocking Buffer (5% BSA/0.09% NaN_3 in Phosphate buffer). Incubate for 1 hr. at room temperature.
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
- 4) Discard the Blocking Buffer. Add 100 μL /well of epitope-tagged control protein (His-DDDDK-V5-HA-Myc-monomeric Azami-Green) in Assay diluent (20 mM HEPES/1% BSA/0.135M NaCl) to each well.
- 5) Incubate for 1 hr. at room temperature.
- 6) Wash the plate with PBS-T [0.05% Tween-20 in PBS] (4 times).
- 7) Add 100 μL /well of Anti-V5-tag mAb-Biotin (MBL; code no. M215-6) diluted with Assay diluent as suggested in the **APPLICATIONS**. Incubate for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the plate with PBS-T (4 times).
- 9) Add 100 μL /well of 1:40,000 Streptavidin-Horseradish Peroxidase (GE Healthcare; code no. RPN4401) diluted with Assay diluent. Incubate for 30 min. at room temperature.
- 10) Wash the plate with PBS-T (4 times).
- 11) Add 100 μL /well of substrate solution (ex. TMB). Incubate for 30 min. at room temperature.
- 12) Add 100 μL /well of stop solution (ex. 1 M H_2SO_4).
- 13) Read at A450 /620.



ELISA for measurement of V5-tagged protein

Sample:

His-DDDDK-V5-HA-Myc-monomeric Azami-Green

Capture antibody:

Anti-His-tag mAb (MBL; code no. D291-3)

Anti-HA-tag mAb (MBL; code no. M132-3)

Anti-Myc-tag mAb (MBL; code no. M192-3)

Anti-DDDDK-tag mAb (MBL; code no. M185-3L)

Detector antibody:

Anti-V5-tag mAb-Biotin (MBL; code no. M215-6)