

Anti-V5-tag mAb-HRP-Direct

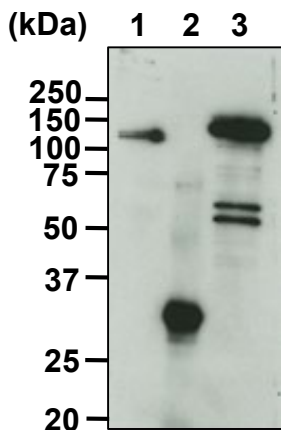
CODE No.	M215-7
CLONALITY	Monoclonal
CLONE	OZA3
ISOTYPE	Mouse IgG2b κ
QUANTITY	100 μ L
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Carrier protein conjugated synthetic peptide, GKPIPPLLGLDST (V5-tag)
FORMULATION	PBS/Preservative/Stabilizer
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at 4°C.
APPLICATION-CONFIRMED	
<u>Western blotting</u>	1:1,000

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

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

SDS-PAGE & Western blotting

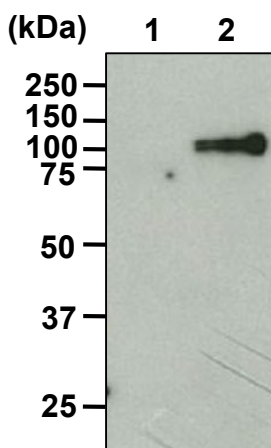
- 1) Mix the sample with equal volume of Laemmili's sample buffer.
- 2) Boil the sample for 2-3 min. and centrifuge. Load the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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 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) 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.
- 9) 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 (20 μ L/lane)
- Lane 2: V5-tagged GFP (25 ng/lane)
- Lane 3: V5-tagged β -galactosidase/293T (10 μ L/lane)

Immunoblotted with Anti-V5-tag mAb-HRP-DirecT (M215-7)



Western blot analysis of V5-tagged protein

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

Immunoblotted with Anti-V5-tag mAb-HRP-DirecT (M215-7)