

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

Smart-IP Series

Anti-V5-tag mAb-Magnetic Agarose

CODE No.	M167-10
CLONALITY	Monoclonal
CLONE	1H6
ISOTYPE	Mouse IgG2a κ
QUANTITY	20 tests (Gel: 200 μ L)
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Carrier protein conjugated synthetic peptide, GKPIPPLLGLDST (V5-tag)
FORMULATION	400 μ g of antibody is covalently coupled to 200 μ L of magnetic agarose gel and provided as 400 μ L gel slurry suspended in PBS/0.1% ProClin 150
STORAGE	This gel slurry is stable for one year from the date of purchase when stored at 4°C.
APPLICATION-CONFIRMED	
<u>Immunoprecipitation</u>	10 μ L of gel/400 μ L of culture sup

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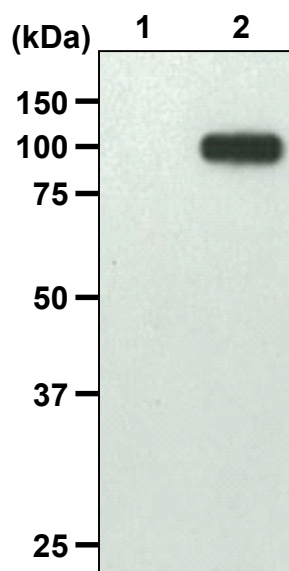
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Immunoprecipitation

- 1) Add magnetic beads as suggested in the **APPLICATION** into 400 μ L of the culture sup. Mix well and incubate with gentle agitation for 30 min. at 4°C.
- 2) Place the tube on the magnetic rack (MBL; code no. 3190) for a few seconds.
- 3) Remove the supernatant.
- 4) Wash the beads 4 times with 1 mL of cold Lysis buffer (place the tube on the magnetic rack for a few seconds).
- 5) Resuspend the magnetic beads in 50 μ L of Laemmli's sample buffer, boil for 3 min., and place the tube on the magnetic rack for a few seconds.
- 6) Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) and carry out 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% 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) 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) PBS for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 10) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3 times).
- 11) Wipe excess buffer on the membrane, 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.
- 12) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual settings. The condition for exposure and development may vary.



Immunoprecipitation of V5-tagged TPO

Lane 1: Insect medium (Negative control)
Lane 2: V5-tagged TPO in insect medium

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