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

*Smart-IP Series*

# Anti-V5-tag mAb-Magnetic Agarose

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| **CODE No.** | M167-10 |
| **CLONALITY** | Monoclonal |
| **CLONE** | 1H6 |
| **ISOTYPE** | Mouse IgG2a κ |
| **QUANTITY** | 20 tests (Slurry: 400 µL) |
| **SOURCE** | Purified IgG from hybridoma supernatant |
| **IMMUNOGEN** | Carrier protein conjugated synthetic peptide, GKPIPNPLLGLDST (V5-tag) |
| **FORMULATION** | 400 µg of antibody is covalently coupled to 400 µL of magnetic agarose 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**

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| Immunoprecipitation | 20 µL of slurry/400 µL of culture sup |

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

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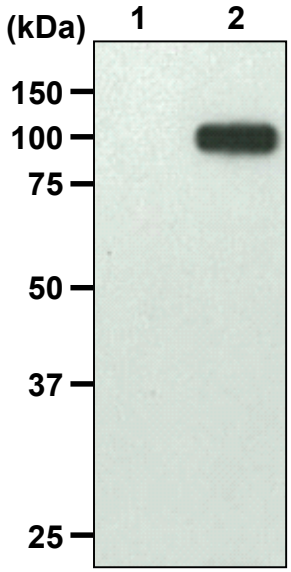
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

## Immunoprecipitation

1) Add magnetic beads as suggested in the **APPLICATION** into 400 µL 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 µL of Laemmli's sample buffer, boil for 3 min., and place the tube on the magnetic rack for a few seconds.
6) Load 10 µL 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/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.
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)