

Smart-IP Series

# Anti-Multi Ubiquitin mAb-Magnetic Beads

**CODE No.** D058-11  
**CLONALITY** Monoclonal  
**CLONE** FK2  
**ISOTYPE** Mouse IgG1  $\kappa$   
**QUANTITY** 20 tests (Slurry: 1 mL)

**SOURCE** Purified IgG from mouse ascites fluid  
**FORMULATION** 5 mg magnetic beads in 1 mL PBS/0.1% BSA/0.09% NaN<sub>3</sub>

\*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.

**STORAGE** This beads suspension is stable for one year from the date of purchase when stored at 4°C.

## APPLICATION-CONFIRMED

Immunoprecipitation 50  $\mu$ L of beads slurry/sample

## SPECIES CROSS REACTIVITY on IP

| Species    | Human | Mouse      | Rat        | Hamster    |
|------------|-------|------------|------------|------------|
| Cells      | 293T  | Not tested | Not tested | Not tested |
| Reactivity | +     |            |            |            |

**REFERENCES**  
1) Takada, K., *et al.*, *Eur. J. Biochem.* **233**, 42-47 (1995)  
2) Fujimuro, M., *et al.*, *FEBS Lett.* **349**, 173-180 (1994)

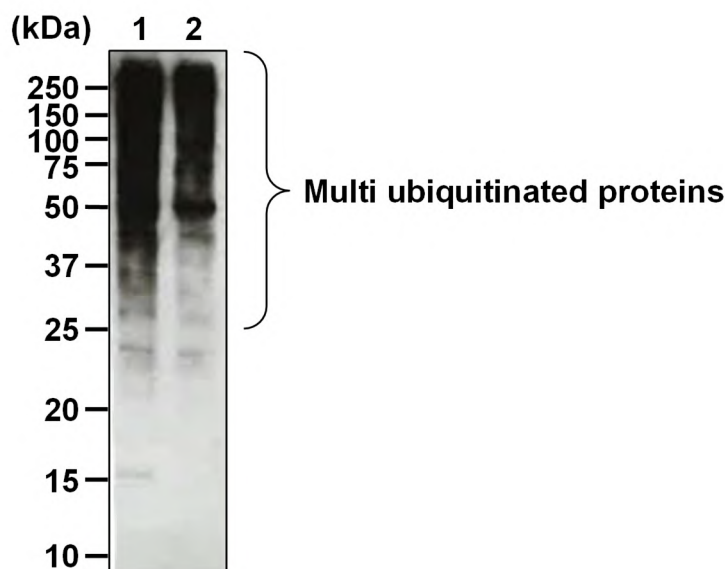
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

## **Immunoprecipitation**

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend with 1 mL of Extraction buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40].
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Add antibody-conjugated magnetic beads as suggested in the **APPLICATION** into 300  $\mu$ L of the supernatant prepared in step 2). Mix well and incubate with gentle agitation for 30 min. at 4°C.
- 4) Place the tube on the magnetic rack (MBL, code no. 3190) for a few seconds.
- 5) Remove the supernatant.
- 6) Wash the bead pellet 3 times with 1 mL of Wash buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] (place the tube on the magnetic rack for a few seconds).
- 7) Elute the multi-ubiquitinated protein by incubation with 20  $\mu$ L of Laemmli's sample buffer (2-ME free) or 0.15 M Glycine-HCl (pH 2.3) for a few minutes at room temperature.
- 8) Place the tube on the magnetic rack for a few seconds and transfer the 10  $\mu$ L of supernatant to another tube.
- 9) Add 10  $\mu$ L of Laemmli's sample buffer and boil for 2 min.
- 10) Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) and carry out electrophoresis.
- 11) 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% methanol). See the manufacturer's manual for precise transfer procedure.
- 12) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 13) Incubate the membrane with 1  $\mu$ g/mL of Anti-Multi Ubiquitin mAb (MBL, code no. D058-3) 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.)
- 14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
- 15) Incubate the membrane with 1:10,000 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.
- 16) Wash the membrane with PBS-T (5 min. x 3).
- 17) 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.
- 18) Expose to an X-ray film in a dark room for 30 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



### ***Immunoprecipitation of multi ubiquitinated protein from 293T***

<Elution buffer>

Lane 1: Laemmli's sample buffer (2-ME free)

Lane 2: 0.15 M Glycine-HCl (pH 2.3)

Immunoblotted with Anti-Multi Ubiquitin mAb (MBL, code no. D058-3)