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

MBL
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Smart-IP Series

Anti-Multi Ubiquitin mAb-Magnetic Beads

CODE No. D058-11

CLONALITY Monoclonal
CLONE FK2
ISOTYPE Mouse IgG1 κ
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_3
*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 µL 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)

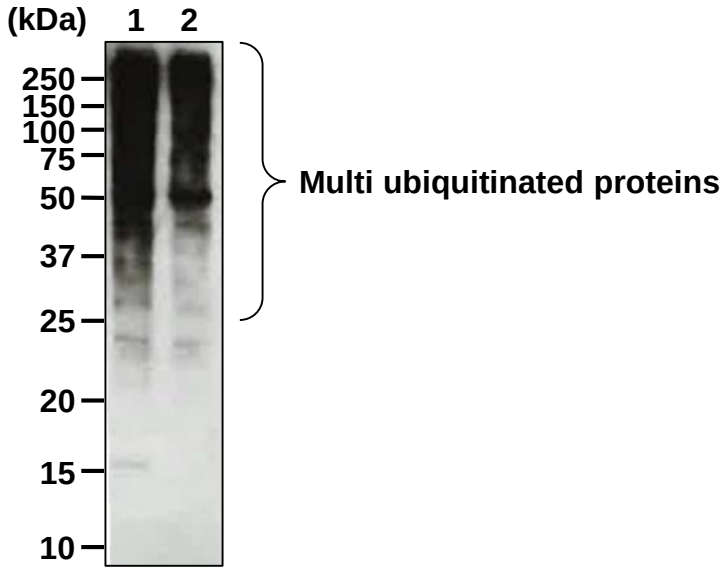
For more information, please visit our web site **https://ruo.mbl.co.jp/**.

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

Immunoprecipitation

1) Wash 1 x 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 µL 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 µL 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 µL of supernatant to another tube.
9) Add 10 µL of Laemmli's sample buffer and boil for 2 min.
10) Load 10 µL 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^2 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 µg/mL of Anti-Multi Ubiqitin 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)