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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-Multi Ubiquitin mAb-Agarose

Code No.	Clone	Subclass	Quantity
D058-8	FK2	Mouse IgG1 κ	Gel: 200 µL

BACKGROUND: Ubiquitin is a polypeptide of 76 amino acid residues, and widely distributed protein in eukaryotic cells. This protein is also highly conserved among eukaryotic cells. Recently several reports showed that intracellular abnormal and short-lived proteins are degradated through an ubiquitin dependent proteolytic pathway. In the ubiquitin dependent pathway, a target protein is tagged with multi-ubiquitin molecules.

SOURCE: This antibody was purified from ascites fluid using protein A agarose. This hybridoma (clone FK2) was established by fusion of mouse myeloma cell with Balb/c mouse splenocyte immunized with a crude poly-ubiquitin-lysozyme.

FORMULATION: 500-600 µg of anti-Multi Ubiquitin monoclonal antibody covalently coupled to 200 µL of agarose gel and provided as a 50% gel slurry suspended in PBS containing preservative (0.1% ProClin 150) for a total volume of 400 µL.

STORAGE: This antibody is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: It is reported clone FK2 recognizes K29-, K48-, K63-linked poly ubiquitinylated and mono ubiquitinylated proteins but not free ubiquitin in on Immunoprecipitation.

APPLICATION:

Immunoprecipitation; 20 µL of gel slurry/300 µL of cell extract from 3 x 10^6 cells

*Please refer to the data sheet (MBL code no. D058-3) for other applications.

Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Raji	Not tested	Not tested
Reactivity on IP	+		

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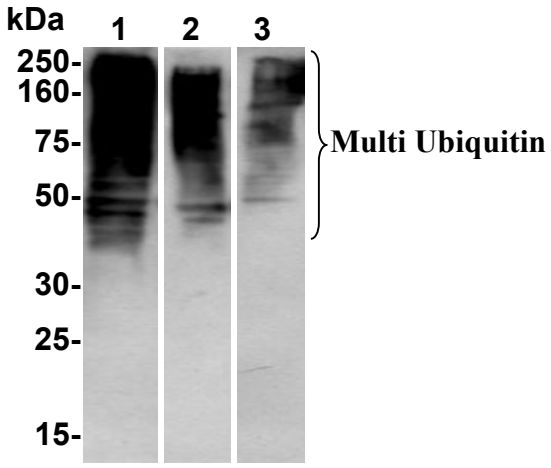
Clone FK2 is used in these references.



Immunoprecipitation of Multi Ubiquitin from Raji cells with D058-8. *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D058-3.*

Sample buffer
Lane 1: SDS-PAGE Buffer (2-ME free)
Lane 2: 170 mM Glycine-HCl (pH 2.3)
Lane 3: 1.5M $MgCl_2$, 50mM Tris-HCl (pH 7.5) 150mM NaCl, 0.05% NP-40

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

PROTOCOL:

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add agarose as suggested in the **APPLICATION** into 300 µL of cell extract. Mix well and incubate with gentle agitation for 30-120 minutes at room temperature.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Add 20 µL of Laemmli's sample buffer, centrifuge the tube for 5 minutes, transfer the supernatant to another tube, and boil for 5 minutes.
6) Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
7) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour 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) for 1 hour at room temperature, or overnight at 4°C.
9) Incubate the membrane with 1 µg/mL of Anti-Multi Ubiquitin mAb (MBL; code no. D058-3) diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
11) 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 hour at room temperature.
12) Wash the membrane with PBS-T (5 minutes x 3 times).
13) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
14) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
15) Expose to an X-ray film in a dark room for 3 minutes.
16) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Raji)

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