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MBL
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MONOCLONAL ANTIBODY

Anti-Multi Ubiquitin mAb

Code No.	Clone	Subclass	Quantity	Concentration
D058-3	FK2	Mouse IgG1 κ	100 µL	1 mg/mL

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: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: It is reported clone FK2 recognizes K29-, K48-, K63-linked poly ubiquitinylated and mono ubiquitinylated proteins but not free ubiquitin in on Western blotting in the reference number 3) and 4).

APPLICATIONS:

Western blotting; 1-5 µg/mL
Immunoprecipitation; Not tested*
*D058-8 is suitable for this application.
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1), 3)-6), 9), 12) and 14).
Immunohistochemistry; Not tested
Flow cytometry; Not tested
ELISA; Not tested*
*It is reported that clone FK2 can be used in this application in the reference number 17).

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse*	Rat	Others*
Cell	Raji	Not tested	Not tested	Not tested
Reactivity on WB	+			

*It is reported that this antibody can be used in mouse[1)-2), 5), 7), 10)], yeast[11), 15)] and monkey[12)].

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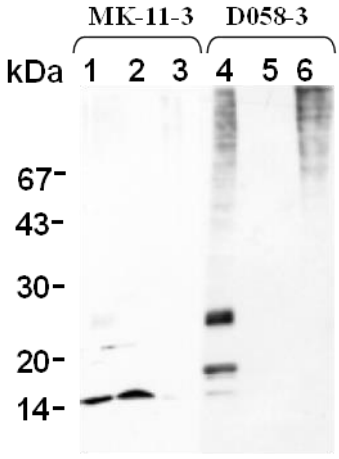

Western blotting analysis of multi ubiquitin expression in Raji cells (1, 4), free ubiquitin (2, 5) and PPUb4 (3, 6) using MK-11-3 (1-3, Anti-Ubiquitin mAb) or D058-3 (4-6).*
**PPUb4: partially purified multi-ubiquitin chains in ubiquitin-protein conjugates*

PROTOCOL:

SDS-PAGE & Western blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] 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. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) 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% methanol). See the manufacture's manual for precise transfer procedure.
6) 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.
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) Incubate the membrane with 1:10,000 of 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.
10) Wash the membrane with PBS-T (5 minutes x 6).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. 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 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Raji, PPUb4*)

*PPUb4; partially purified multi-ubiquitin chains in ubiquitin-protein conjugates

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