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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-Multi Ubiquitin mAb

Code No.	Clone	Subclass	Quantity	Concentration
D071-3	FK1	Mouse IgM	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. There are several reports showed that intracellular abnormal and short-lived proteins are degraded 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 mouse ascites fluid by affinity chromatography. This hybridoma (clone FK1) was established by fusion of mouse myeloma cell with Balb/c mouse splenocyte immunized with the crude poly-ubiquitin-lysozyme.

FORMULATION: 100 µg IgM 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 FK1 recognizes K29-, K48-, K63-linked poly-ubiquitinylated proteins but not mono-ubiquitinylated proteins or free ubiquitin on Western blotting in the reference number 1).

APPLICATIONS:

Western blotting; 1-5 µg/mL
Immunoprecipitation; Not tested
Immunocytochemistry; Not tested
Immunohistochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

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

REFERENCES:

1) Morris, J. R., and Solomon, E., *Hum Mol Genet.* **13**, 807-817 (2004)
2) Seino, H., *et al., Mol Cell Biol.* **23**, 3497-3505 (2003)
3) Lyakhovich, A., *et al., Mol Cell Biol.* **2**3, 2463-2475 (2003)
4) Takeuchi, J., *et al., Mol Cell Biol.* **19**, 6575-6584 (1999)
5) Takada, K., *et al., Eur J Biochem.* **233**, 42-47 (1995)
6) Fujimuro, M., *et al., FEBS Letters.* **349**, 173-180 (1994)

Clone FK1 is used in reference number 1) - 4) and 6).

RELATED PRODUCTS:

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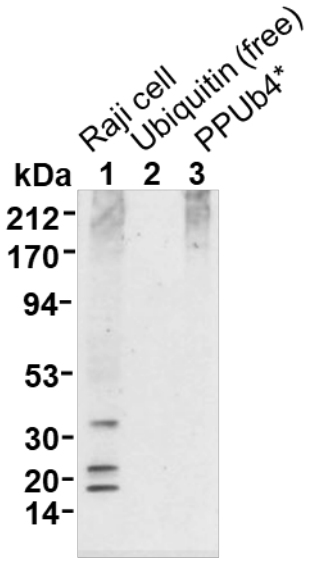
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 cold 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 3 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 PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (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 (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 1 minute.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Raji, PPub4)



Western blotting analysis of multi-ubiquitin using D071-3.

* PPub4; partially purified multi-ubiquitin chains in ubiquitin-protein conjugates