

MONOCLONAL ANTIBODY

Anti-Ubiquitin mAb

Code No.	Clone	Subclass	Quantity	Concentration
MK-11-3	1B3	Mouse IgG1	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 a 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 by ammonium sulfate precipitation and protein A agarose. This hybridoma (clone 1B3) was established by fusion of mouse myeloma cell P3U1 with F1 mouse (C57BL/6 x Balb/c) splenocyte immunized with the bovine erythrocyte ubiquitin.

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: This antibody reacts with human and bovine erythrocyte ubiquitin on Western blotting. Clone 1B3 does not react with multi ubiquitin. Clone 1B3 and 2C5 (MBL; code no. MK-12-3) recognize different epitope sites each other.

APPLICATIONS:

Western blotting; 5 µg/mL for chemiluminescence detection system

Immunoprecipitation; Not tested

Immunohistochemistry; Reference 2) and 5)

Immunocytochemistry; Reference 1) and 9)

Flow cytometry; Not tested

Immunoelectron microscopy; Reference 2)

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

INTENDED USE:

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

SPECIES CROSS REACTIVITY:

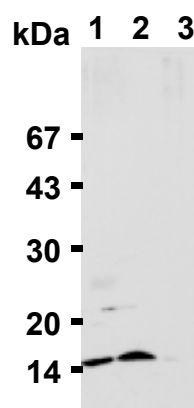
Species	Human	Mouse*	Rat	Bovine**
Cell	Raji		Not tested	
Reactivity on WB	+			

It is reported in the reference number *2) and **15).

REFERENCES:

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- 8) Zhang, H. F., *et al.*, *Cancer Res.* **64**, 1114-1121 (2004) [WB]
- 9) Nakamichi, I., *et al.*, *Mol. Biol. Cell* **13**, 3441-3451 (2002) [IC]
- 10) Hatakeyama, S., *et al.*, *J. Biol. Chem.* **276**, 33111-33120 (2001) [WB]
- 11) Ageta, H., *et al.*, *J. Biol. Chem.* **276**, 15893-15897 (2001) [WB]
- 12) Yamanaka, A., *et al.*, *Mol. Biol. Cell* **11**, 2821-2831 (2000) [WB]
- 13) Hattori, K., *et al.*, *J. Biol. Chem.* **274**, 29641-29647 (1999) [WB]
- 14) Hatakeyama, S., *et al.*, *PNAS* **96**, 3859-3863 (1999) [WB]
- 15) Mitsui, A., and Sharp, P. A., *PNAS* **96**, 6054-6059 (1999) [WB]
- 16) Shirane, M., *et al.*, *J. Biol. Chem.* **274**, 28169-28174 (1999) [WB]
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Clone 1B3 is used in these references.



Western blot analysis of ubiquitin expression in Raji (1), free ubiquitin (2) and PPUb4* (3) using MK-11-3.

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

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/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). 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 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 (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 3 minutes.
- 14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Raji, free ubiquitin)

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