

MONOCLONAL ANTIBODY

# Anti-Ubiquitin

Code No.	Clone	Subclass	Quantity	Concentration
MK-12-3	2C5	Mouse IgG1	100 µg	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 ascites fluid using protein A agarose. This hybridoma (clone 2C5) 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, mouse, rat and bovine ubiquitin on Western blotting. Clone 2C5 does not react with multi ubiquitin. Clone 2C5 and 1B3 (MBL; code no. MK-11-3) recognize different epitope sites each other.

**APPLICATIONS:**

Western blotting: 5 µg/mL for chemiluminescence detection system

Immunoprecipitation: Not tested\*

\*It is reported that 2C5 can be used in this application in the reference number 4).

Immunohistochemistry: Not tested

Immunocytochemistry: Not tested\*

\*It is reported that 2C5 can be used in this application in the reference number 3).

Flow cytometry: Not tested

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

**INTENDED USE:**

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

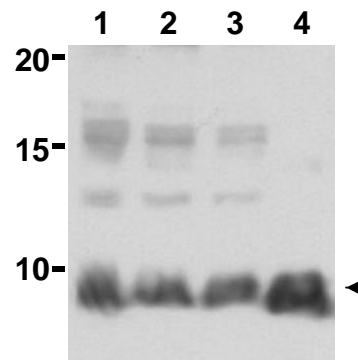
**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat	Bovine
Cells	Jurkat, HeLa, Raji, HL-60	WR19L	PC12	erythrocyte
Reactivity on WB	+	+	+	+

**REFERENCES:**

- 1) Ishioka, T., *et al.*, *Genes Cells* **12**, 735-744 (2007)
- 2) Ott, D. E., *et al.*, *J. Virol.* **77**, 3384-3393 (2003)
- 3) Sutovsky, P., *et al.*, *Biol. Reprod.* **63**, 582-590 (2000)
- 4) Hiyama, H., *et al.*, *J. Biol. Chem.* **274**, 28019-28025 (1999)

Clone 2C5 is used in these references.



**Western blot analysis of ubiquitin expression in Raji (1), HeLa (2), HL-60 (3) and ubiquitin purified protein (4) using MK-12-3.**

**PROTOCOL:**

**SDS-PAGE & Western Blotting**

- 1) Wash cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at  $1 \text{ mA/cm}^2$  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.
- 4) 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.

- 5) 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 the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) 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.
- 8) Wash the membrane with PBS-T (10 minutes x 3 times).
- 9) Drain excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose to an X-ray film in a dark room for 3 minutes.
- 12) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting: Jurkat, Raji, HeLa, HL-60, WR19L, PC12)

## **RELATED PRODUCTS:**

### Antibody

MK-11-3	anti-Ubiquitin (1B3)
D058-3	anti-Multi Ubiquitin (FK2)
D058-8	anti-Multi Ubiquitin-Agarose (FK2)
D071-3	anti-Multi Ubiquitin (FK1)
PM023	anti-NEDD8 (polyclonal)
M113-3	anti-SUMO-1 (5B12)
M114-3	anti-SUMO-2/3 (1E7)
M114-A48	anti-SUMO-2/3-Alexa Fluor <sup>®</sup> 488 (1E7)
M162-3	anti-p62/SQSTM1 (5F2)
PM045	anti-p62/SQSTM1 (polyclonal)

### Kit

CY-7053	CycLex <sup>®</sup> Poly-Ubiquitinated Protein ELISA Kit
CY-7001	CycLex <sup>®</sup> Poly-Ubiquitinated Protein Enrichment & Detection Kit

### Isotype control

M075-3	Mouse IgG1 isotype control (2E12)
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