V.6.0

Anti-Ubiquitin Mouse MAb

Cat. # AUB01-XL

Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	4 x 500 µl when reconstituted
Validated applications:	WB, IF
Species reactivity:	Wide range of species
Host/Isotype:	Mouse/IgG1
Clone:	P4D1

Background Information

Ubiquitin (Ub) and ubiquitin-like proteins (Ubls, e.g. SUMO, Nedd) are a group of approximately 15 proteins that have a molecular weight of around 8 kD. During the ubiquitination process, these are conjugated via activating (E1), conjugating (E2) and ligating (E3) enzymes to lysines of a target protein (1). Mammalian cells express over 600 potential ubiquitin ligases which exceeds that of the kinase superfamily of PTM proteins (2).

One function of ubiquitination is to target proteins for proteosomal degradation. This role can range from a general housekeeping function that clears miss folded proteins from a cell to involvement in tightly regulated spatio-temporal cell signaling events (1). An emerging function of ubiquitination is its ability to activate proteins via the creation of unique protein:protein interactions (3). In common with many other PTMs, ubiquitination is reversible. Ubiquitin-specific proteases (USPs or DUBs) remove ubiquitins from target proteins (4). The reversible nature of ubiquitination further enhances the potential of this PTM to dynamically regulate protein function.

Material

Anti-ubiquitin antibody is a mouse monoclonal antibody. The hybridoma has been licensed by Cytoskeleton from Fred Hutchinson Cancer Research Center. The antibody was raised against full length bovine ubiquitin and recognizes polyubiquitin and free ubiquitin (Fig 1). Ubiquitin is a highly conserved protein and AUB01 is predicted to recognize ubiquitin from a wide range of species (5). AUB01 is purified by Protein G affinity chromatography and is supplied as a lyophilized white powder.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder to the bottom of the tube.

Reconstitute each tube in 500 µl of room temperature 50% glycerol and store at –20°C. Final buffer composition is 50 mM PIPES pH 7.0, 1% sucrose, 0.5% dextran, 50% glycerol.

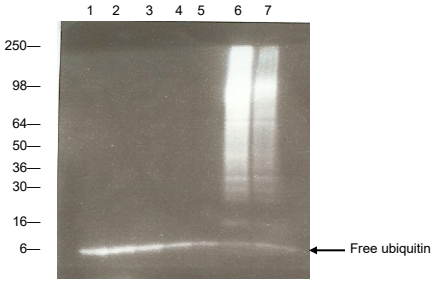
When stored and reconstituted as described, the product is stable for 6 months at 4°C. NOTE: We recommend adding an antibacterial such as sodium azide (0.02% final concentration) to prevent bacterial contamination of the antibody stock.

Applications

Western Blot (WB) Applications

Use as indicated below at 1:500 dilution, sufficient for 1000 ml of working strength Ab.

Figure 1 : Western blot applications



Legend: AUB01 was used at a 1:500 dilution following the recommended Western blot protocol (see below). Bovine thymus ubiquitin was run as follows; Lane 1-50 ng, Lane 2-25 ng. Lane 3–12.5 ng, Lane 4-6.25 ng, Lane 5-3.12 ng. Lanes 6 & 7 represent 20 ug of Swiss 3T3 cell lysate from cells treated for 5h with 10 µM MG132 (Lane 6) or untreated cells (Lane 7). Arrow indicates free ubiquitin band (8 kD), higher molecular weight bands are ubiquitinated proteins.

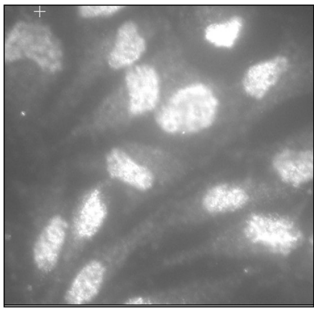
Western Blot Method:

1. Run protein samples and control samples in SDS-PAGE.
2. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, and 15% methanol) for 15 min at room temperature prior to electro-blotting.
3. Transfer the protein to a PVDF membrane for 45 min at 75 V.
4. Wash the membrane with TBST for 10 min. with constatnt agitation (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
5. Block the membrane surface with 5% nonfat-dry milk in TBST for 30 min at room temperature with constant agitation.
6. Incubate the membrane with a 1:500 dilution of anti-ubiquitin antibody, diluted in TBST, for 1-2 h at room temperature or overnight at 4°C with constant agitation.
7. Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
8. Incubate the membrane with an appropriate dilution (e.g., 1:20,000) of anti-mouse secondary antibody (e.g., goat anti-mouse HRP conjugated IgG from Jackson Labs., Cat. # 115-035-068) in TBST/0.5% non-fat milk for 60 min at room temperature with constant agitation.
9. Wash the membrane 6 times in TBST for 10 min each with constant agitation.
10. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

Immunofluorescence (IF) Applications

Use as indicated below at 1:500 dilution, sufficient for 100 ml of working strength Ab, approx. 1000 slides.

Figure 2: IF of HeLa cells



Legend: HeLa cells were stained and visualized by fluorescence microscopy as described in the IF method below. Ubiquitin targeted cytoplasmic and nuclear proteins and free ubiquitin were stained using AUB01 at 1:500 dilution.

IF Method

1. Plate HeLa cells at 1 x 10^5/ml on acid washed coverslips in tissue culture dish with DMEM media containing 10% FBS.
2. Allow cells to grow for 96 h to reach 60% confluency.
3. Permeabilize cells with 50 µg/ml digitonin in 20 mM HEPES pH7.4, 100 mM sodium acetate, 1 mM EGTA, 2 mM DTT, 10 mM NEM for 5 minutes.
4. Fix cells with 2% paraformaldehyde in PBS for 30 minutes.
5. Wash coverslips three times in PBS at room temperature over a 5 min period.
6. Place coverslips cell side up on parafilm and apply 100-200 µl of AUB01 solution (1:500 in PBS/2% BSA).
7. Incubate at room temperature for 45 min.
8. Wash coverslips three times in PBS at room temperature over a 5 min period.
9. Apply 100-200 µl of fluorescently-labeled anti-mouse secondary antibody at manufacturer's recommended dilution. For example, we use fluorescently-labeled goat anti-mouse at 1:200 dilution in PBS/2% BSA.
10. Incubate at room temperature for 30 min.
11. Wash coverslips three times in PBS at room temperature over a 5 min period.
12. Place coverslips, cell side down, on glass slide with mounting media (e.g., EMS, Cat# 17987-10) and observe cells under fluorescence microscope.

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Immunoprecipitation (IP) Applications
The antibody has been tested in IP applications and is not recommended.

References
1) Grabbe, C. et al. 2011. The spatial and temporal organization of ubiquitin networks. Nat. Rev. Mol. Cell. Biol. 12:295-307.
2) Deshaies R.J. & Joazeiro, C.A. 2009. RING domain E3 ligases. *Ann. Rev. Biochem.* **78**: 399-434.
3) Lomeli, H. & Vazquez. 2011. Emerging roles of the SUMO pathway. Cell Mol. Life Sci. 68:4045-4064.
4) Faesen, A.C. et al. 2012. The role of UBL domains in ubiquitin specific proteases. Biochem. Soc. Trans. 40:539-545.
5) Zuin, A. et al. 2014. Ubiquitin signaling: Extreme conservation as a source of diversity. *Cells 3:690-701*.

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