V.5.0

Anti-Ubiquitin Mouse Mab-HRP Labeled

Cat. # AUB01-HRP-S
Lot:
Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	25 µl when reconstituted
Validated applications:	WB
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 conjugated to HRP 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.

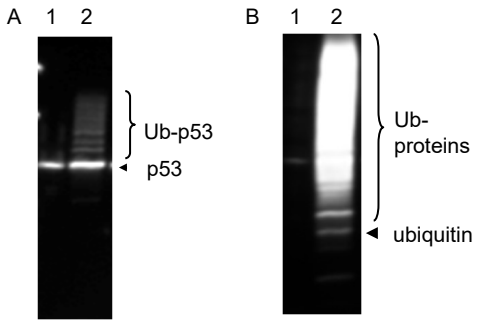
Reconstitute the tube in 25 µl of room temperature 50% glycerol and store at –20°C. Final buffer composition is 50 mM PIPES pH 7.0, 1% sucrose, 0.5% dextran, 50% glycerol.

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 1:4000 dilution, sufficient for 100 ml of working strength Ab.

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 or overnight at 20V.
4. Wash the membrane with TBST for 10 min. with constant 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 1:4000 dilution of anti-ubiquitin-HRP antibody, diluted in TBST/3% milk, for 1-2 h at room temperature or overnight at 4°C with constant agitation.
7. Wash the membrane 6 times in TBST for 10 min each with constant agitation.
8. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

Figure 1 : Western blot applications



HeLa cells were grown to 70% confluency and harvested by lysis in BlastR™ buffer. Lysates (500 µg per assay) were treated as outlined in the Signal-Seeker™ Ubiquitination Enrichment manual. The western blot (A) was probed with an anti-p53 antibody and re-probed (B) with anti-ubiquitin-HRP antibody. Lane A1, 2% input lysate; Lane A2, IP from 500 µl of HeLa lysate enriched using ubiquitination affinity beads. Ubiquitinated p53 is clearly visible in the IP lane which agrees with published data (6). Western blot (B) shows blot (A) re-probed with anti-ubiquitin-HRP antibody. Lane 1 shows a slight signal at the position of p53 as the blot was not stripped prior to re-probing.

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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