V.1.0

Anti-Acetyl Lysine Mouse Monoclonal Antibody Horseraddish peroxidase conjugates

Cat. # AAC03-HRP-S

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

Form:	Lyophilized powder
Amount of material:	1 x 25 µl when reconstituted
Validated applications:	WB
Species reactivity:	All
Host/Isotype:	Mouse/IgG1
Clone:	19C4B2.1

Background Information

Acetylation of proteins can occur as a co-translational or post-translational modification (PTM) (1). Co-translational acetylation occurs at the N-terminal of approximately 85% of mammalian proteins, it is irreversible and is thought to be important in protein stability, localization and synthesis (1). Post-translational acetylation occurs on the epsilon amino group of lysine residues as a reversible and highly dynamic PTM that is known to be a key regulator in multiple cellular events, including chromatin structure, transcription, metabolism, signal transduction and cytoskeletal regulation (2-3). To date over 4,000 proteins have been identified as targets for PTM acetylation (3).

Material

AAC03-HRP is a mouse monoclonal antibody. The antibody was raised against a proprietary mixture of acetylated proteins designed to optimize acetyl lysine recognition in a wide range of sequence contexts. The antibody has been shown to recognize a broad range of acetylated proteins, including acetylated tubulin, histones, and chemically acetylated bovine serum albumin (Fig. 1). AAC03-HRP is supplied as a lyophilized white powder. Each Lot of antibody is quality controlled to provide high batch-to-batch consistency. The Lot specific µg per tube can be found in the Lot specific COA documents at www.cytoskeleton.com.

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 at the bottom of the tube.

Reconstitute each tube with 25 µl of 50% glycerol (room temperature). We do not recommend using 50% glycerol at 4°C as this can cause the lyophilized antibody to stick to the pipet tip during resuspension. Store reconstituted antibody at –20°C. Final buffer composition is 200 mM PIPES, 50% glycerol, 5% sucrose,1% dextran and 10mg/ml BSA.

When stored and reconstituted as described, the product is stable for 12 months at -20°C. **NOTE: Sodium azide is an irreversible inhibitor of HRP. Do not add sodium azide to APY03-HRP antibody**.

Applications

Western Blot (WB) Applications

Use as indicated below at 1:3000-1:6000 dilution, sufficient for 75-150 ml of working strength Ab.

Western Blot Method:

1. Run protein samples and control samples in SDS-PAGE.
2. We recommend running 30 µg of TSA/nicotinamide-treated Cos-7 cell lysate as a control.
3. 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.
4. Transfer the protein to a PVDF membrane for 60 min at 70 V.
5. Wash the membrane once with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
6. The membrane may be left in TBST overnight at 4°C if convenient.
7. Block the membrane surface with 3% nonfat-dry milk in TBST for 60 min at room temperature with constant agitation.
8. Incubate the membrane with a 1:3000-1:6000 dilution of anti-acetyl lysine antibody, diluted in 3% nonfat-dry milk in TBST, for 1-2 h at room temperature or overnight at 4°C with constant agitation.
9. Rinse the membrane five times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
10. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

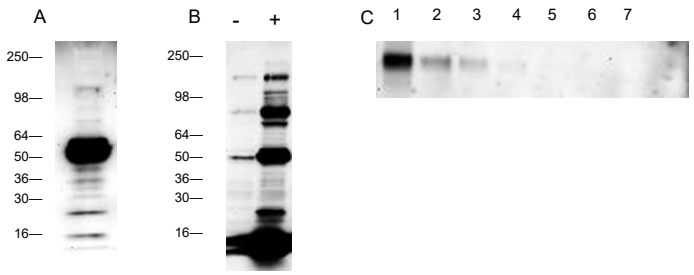

Fig 1: **Utilization of AAC03-HRP for western blotting. A**: Murine tissue extract, 30 µg brain extract. **B**: 30 µg of Cos-7 cell lysate treated with TSA and nicotinamide (+) or untreated (-). Strongly enhanced bands at 55 and 14-16 kDa in TSA-treated lysate correspond to acetylated tubulin and histone proteins, respectively. **C**: Titration of acetylated BSA. Lanes 1-5 contain 0.5, 0.1, 0.05, 0.01, and 0.005 ng Ac-BSA, lanes 6-7 contain 500 and 1000 ng non-acetylated BSA, respectively. AAC03-HRP was used at a 1:3000 dilution following the recommended Western blot protocol.

References

1 Bogdan P. and Sherman F. 2002. The diversity of acetylated proteins. Genome Biol. 3 (5): reviews 0006.

2 Lundby A. et al. 2012. Proteomic analysis of lysine acetylation sites in rat tissues reveals organ specificity and cellular patterns. Cell Reports 2:419-431.

3 Sadoul K. et al. 2010. The tale of protein lysine acetylation in the cytoplasm. J. Biomed. Biotech. 2011:1-15.

4 Golemis EA et. Al, Protein-Protein Interactions, CSHLP, 2005, p67

Product Citations/Related Products

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Phone: (303) 322.2254 **Fax:** (303) 322.2257
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Technical Support: tservice@cytoskeleton.com