

Anti-Acetyl Lysine Mouse Monoclonal Antibody

Cat. # AAC02-S

Lot:

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, IF, IP, ChIP
Species reactivity:	All
Host/Isotype:	Mouse/IgG2b
Clone:	7B5A1

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). The AAC02 antibody detects acetyl lysine PTMs.

Material

Anti-acetyl lysine antibody AAC02 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). AAC02 was purified by Protein G affinity chromatography and is supplied as a lyophilized white powder.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized antibody 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 in 25µl 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, and 1% dextran.

When stored and reconstituted as described, the product is stable for 12 months at -20°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 1:500-1:1000 dilution, sufficient for 12.5-25 ml of working strength Ab.

Western Blot Method:

- Run protein samples and control samples in SDS-PAGE.
- We recommend running 30 µg of TSA/nicotinamide-treated Cos-7 cell lysate as a control.
- 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.
- Transfer the protein to a PVDF membrane for 60 min at 70 V.
- Wash the membrane once with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
- The membrane may be left in TBST overnight at 4°C if convenient.
- Block the membrane surface with 3% nonfat-dry milk in TBST for 60 min at room temperature with constant agitation.
- Incubate the membrane with a 1:500-1:1000 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.
- Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
- 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/3% non-fat milk for 60 min shaking a room temp.

- Wash the membrane 5 times in TBST for 10 min each.
- Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

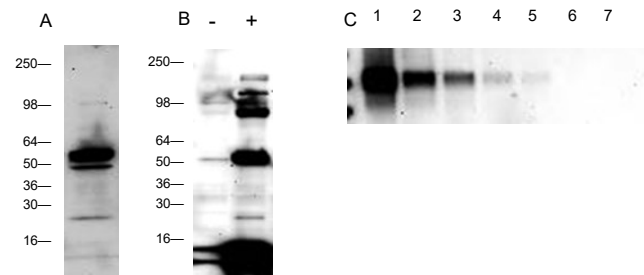


Fig 1: Utilization of AAC02 for western blotting. **A:** Murine tissue extract, 30 µg brain extract. **B:** 30 µg of Cos-7 cell lysate treated with TSA and nicotinamide (+) or untreated (-). Strongly enhanced bands at 55 and 14-16 kDa in TSA-treated lysate correspond to acetylated tubulin and histone 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. AAC02 was used at a 1:500 dilution following the recommended western blot protocol.

Immunoprecipitation (IP) Applications

Use as indicated at 20 µl per IP reaction, sufficient for approximately 1 IP assay.

IP Method

- Add 20 µl of antibody to 500 µl of PBS pH 7.4 in a microfuge tube containing 30 µl of packed Protein G agarose pre-equilibrated in PBS.
- Gently rotate the reaction for 1 h at 4°C.
- Add 500 µl of PBST to the mixture and centrifuge for 1 min at 4°C and 3000 rpm (approx. 960 x g). Addition of the PBST will prevent agarose from sticking to the microfuge tube walls.
- Discard supernatant and wash beads 3X in PBST.
- Add 1-1.5 mg of cell lysate (1-1.5 mg/ml protein concentration) to the beads. The lysate must be prepared in an IP compatible buffer (e.g. BlastR lysis buffer and filter system).
- Gently rotate the reaction at 4°C for 2 h or overnight if convenient.
- Spin down agarose for 1 min 4°C at 3000 rpm (approx. 960 x g).
- Discard supernatant and wash beads with 1ml of IP wash buffer (e.g BlastR wash buffer) at 4°C.
- Repeat wash two more times.
- Resuspend beads in 30 µl of 2X Laemmli buffer (125 mM Tris pH 6.8, 20% glycerol, 4% SDS, 0.005% Bromophenol blue, 5% beta-mercaptoethanol) and boil for 5 min prior to loading on SDS-PAGE for subsequent Western blot analysis.

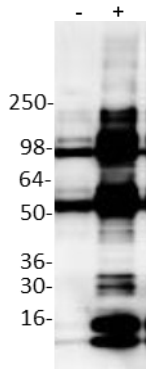


Fig 2: Utilization of AAC02 for Immunoprecipitation. Cos-7 cells were either treated (+) or untreated (-) with TSA (1 μ M) and nicotinamide (1mM) for 6 hours. Cell lysates were prepared in BlastR buffer and filter system and 1 mg of lysate per reaction was used for IP of acetylated proteins. 20 μ l of AAC02 was used per IP reaction. Western blots of immunoprecipitated proteins were developed using AAC03-HRP at 1:3000 dilution.

Immunofluorescence (IF) Applications

Use as indicated below at 1: 500 –1: 1000 dilution, sufficient for 12.5–25ml of working strength Ab.

IF Method (TSA treatment of Swiss 3T3 cells)

1. Plate SWISS 3T3 cells at 1×10^5 /ml on acid washed coverslips in tissue culture dish with DMEM media containing 10% FBS.
2. Allow cells to grow for 24-48 h, then treat one set of coverslips with TSA (1 μ M for 6 h). MitoTracker orange was added to the cells at 100nM for 30 min to stain mitochondria before fixation (optional).
3. Fix cells with 4% formaldehyde for 10 min. Rinse coverslips in PBS.
4. Permeabilize in 0.5% Triton-X for 20 minutes.
5. Rinse coverslips 3 times in PBS.
6. Add blocking buffer (1% BSA/PBST) to coverslips.
7. Incubate at room temperature for 30 min.
8. Wash coverslips 3 times in PBS at room temperature, 5 min per wash.
9. Apply AAC02 (1:500) in blocking solution and incubate at room temp for 1 hr.
10. Wash coverslips 3 times in 0.5% Triton-X.
11. Apply fluorescently-labeled anti-mouse secondary antibody at manufacturer's recommended dilution. For example, we use fluorescently-labeled goat anti-mouse at 1:500 dilution in blocking buffer.
12. Incubate at room temperature for 45 min.
13. Wash coverslips 3 times in PBST at room temperature, 5 min per wash.
14. Add Rhodamine Phalloidin (100nM) in PBS to coverslips and incubate at room temp for 20 min.
15. Wash coverslips 3 times in PBS at room temperature, 5 min per wash.
16. Rinse coverslips briefly in sterile water.
17. Place coverslips on glass slide with mounting media (e.g., EMS, Cat# 17987-10) and observe cells under fluorescence microscope.

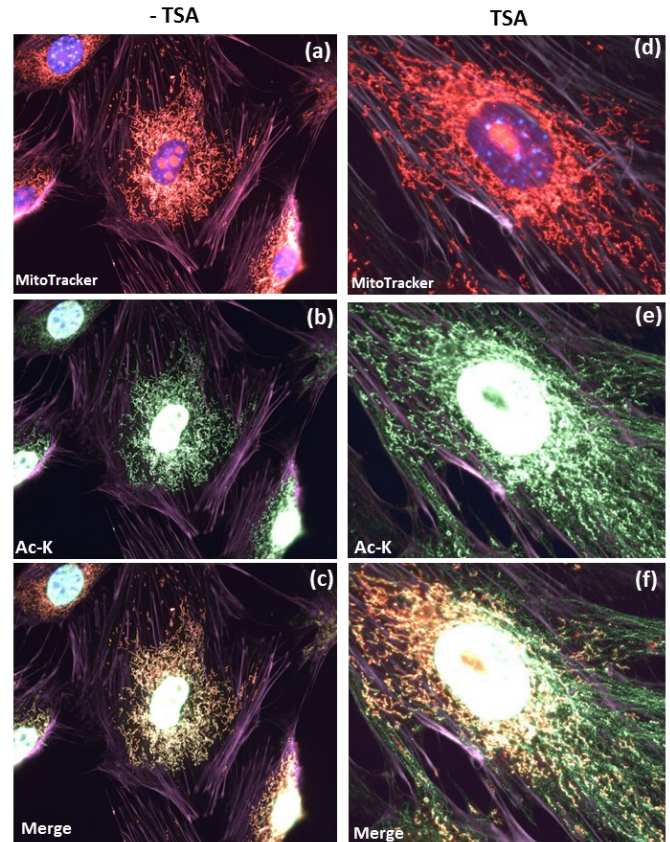


Fig 4: AAC02 detects mitochondrial acetylation. Swiss 3T3 cells, either untreated or treated with TSA (1 μ M for 6h), were stained as described in the method. **(a and d)**: Mitochondria were visualized with MitoTracker orange (Thermo Fisher). **(b and e)**: Acetylated cytoplasmic and nuclear proteins were visualized in green fluorescence. **(c and f)**: Merged image of mitochondrial and acetylation signals. Actin fibers and nuclei were visualized in purple with Rhodamine Phalloidin and blue with DAPI respectively. Note: AAC02 provides broad, pan acetyl-lysine detection including acetylated mitochondrial, nuclear, and cytoplasmic proteins.

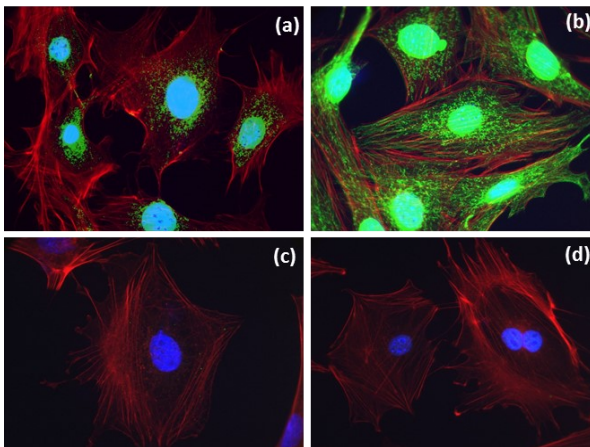


Fig 3: Utilization of AAC02 for Immunofluorescence. Swiss 3T3 cells, untreated (a and c) or treated (b and d) with TSA (1 μ M for 6 h), were stained as described. Acetylated proteins were visualized using a green fluorescent secondary. Actin fibers were visualized using a red Rhodamine Phalloidin and the nucleus was stained with DAPI. The acetylated microtubule network is clearly visible with TSA-treatment, while the green fluorescent nuclear intensity indicate the high abundance of acetylated proteins in the nucleus. In c and d, acetylated BSA (10 μ g/ml) was used to compete for AAC02 binding as an indicator of AAC02 specificity for acetyl-lysine modifications.

Chromatin Immunoprecipitation (ChIP) Application

Use as indicated below at 1:100 dilution, sufficient for 25 ChIP assays of 100 μ l volume.

ChIP Method

1. Sheared and cross-linked chromatin from A431 cells was prepared according to published protocols (4).
2. Approximately 25 μ g of chromatin is used per ChIP assay, the reactions are carried out in the following ChIP buffer: 10 mM Tris pH 8.0, 0.5 mM EGTA, 1.0 mM EDTA, 140 mM NaCl, 1% Triton, 0.1% Na deoxycholate, 0.15% SDS, 1 mM PMSF, 2 μ g/ml pepstatin/leupeptin, and aprotinin.
3. Bring the final volume to 100 μ l in ChIP reaction buffer and add 1 μ l of AAC02 per ChIP reaction. NOTE: it is also recommended to run mouse IgG as a non-specific control reaction (see Ctr mIgG lanes above) as well as a no-antibody input control (see Figure 5).
4. Incubate on a rotator at 4°C for 1-2 h or overnight.
5. Add 20 μ l of a 50% slurry of Protein G beads to all reactions and rotate at 4°C for 2-4 h.
6. Pellet beads by centrifugation at 3000 rpm (approx. 960 x g) for 1 min.
7. Transfer the no-antibody input control supernatant to a fresh tube on ice, this will be processed later and serve as the total input control.
8. Discard all other supernatants.
9. Wash beads five times with 500 μ l of RIPA buffer (10 mM Tris pH 8.0, 1 mM EDTA, 0.5 mM EGTA, 1% Triton, 0.1% SDS, 0.1% Na deoxycholate, 140 mM NaCl, and 1 mM PMSF).
10. Wash beads once with 500 μ l of LiCl buffer (0.25 M LiCl, 0.5% NP-40, 0.5% Na deoxycholate, 1 mM EDTA, 10 mM Tris pH 8.0).
11. Wash beads once with 500 μ l of TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) and resuspend beads in 100 μ l of TE buffer.
12. The immunoprecipitated chromatins bound to the bead samples (and chromatins in the no-antibody input control supernatant) were processed for DNA isolation and PCR analysis according to published protocols (4).

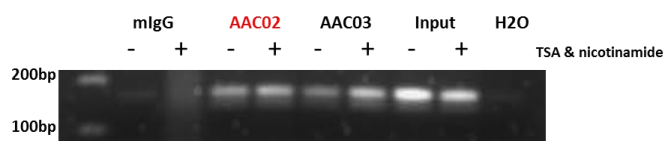


Fig 5: Utilization of AAC02 for ChIP. Chromatin was prepared from A431 cells, either untreated or treated with TSA (1 μ M) and nicotinamide (1mM) for 6 hours. ChIP was performed as described. mIgG: mouse IgG used for ChIP control; AAC02: anti-acetyl lysine antibody used for ChIP; Input: cell lysate prior to ChIP; H2O: Water used as PCR control. The PCR products obtained with GAPDH primers are 166 bp.

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

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