V.4.0

Anti-Acetyl Lysine Mouse Monoclonal Antibody

Cat. # AAC02-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, 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, and1% 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:

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 1:500-1:1000 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.
9. Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
10. 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.
11. Wash the membrane 5 times in TBST for 10 min each.
12. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

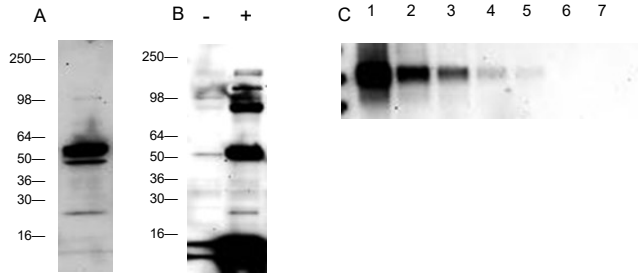

Fig 1: **Utilization of AAC02 for western blotting. A**: Murine tissue extract, 30 µg brain extract. **B**: 30 µg of Cos-7 cell lysate treated with TSA and nicotinamide (+) or untreated (-). Strongly enhanced bands at 55 and 14-16 kDa in TSA-treated lysate correspond to acetylated tubulin and histone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

1. 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.
2. Gently rotate the reaction for 1 h at 4°C.
3. 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.
4. Discard supernatant and wash beads 3X in PBST.
5. 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).
6. Gently rotate the reaction at 4°C for 2 h or overnight if convenient.
7. Spin down agarose for 1 min 4°C at 3000 rpm (approx. 960 x g).
8. Discard supernatant and wash beads with 1ml of IP wash buffer (e.g BlastR wash buffer) at 4°C.
9. Repeat wash two more times.
10. 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.

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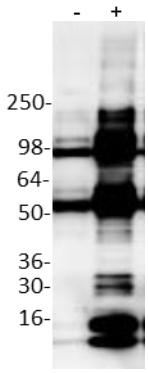

Fig 2: **Utilization of AAC02 for Immunoprecipitation.** Cos-7cells were either treated (+) or untreated (-) with TSA (1 μM) and nicotinamide (1mM) for 6 hours. Cell lysates were prepared in BlastR buffer and filter system and 1 mg of lysate per reaction was used for IP of acetylated proteins. 20μl of 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 x 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 μM for 6 h). MitoTracker orange was added to the cells at 100nM for 30 min to stain mitochondria before fixation (optional).
4. Fix cells with 4% formaldehyde for 10 min. Rinse coverslips in PBS.
5. Permeabilize in 0.5% Triton-X for 20 minutes.
6. Rinse coverslips 3 times in PBS.
7. Add blocking buffer (1% BSA/PBST) to coverslips.
8. Incubate at room temperature for 30 min.
9. Wash coverslips 3 times in PBS at room temperature, 5 min per wash.
10. Apply AAC02 (1:500) in blocking solution and incubate at room temp for 1 hr.
11. Wash coverslips 3 times in 0.5% Triton-X,
12. 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.
13. Incubate at room temperature for 45 min.
14. Wash coverslips 3 times in PBST at room temperature, 5 min per wash.
15. Add Rhodamine Phalloidin (100nM) in PBS to coverslips and incubate at room temp for 20 min.
16. Wash coverslips 3 times in PBS at room temperature, 5 min per wash.
17. Rinse coverslips briefly in sterile water.
18. Place coverslips on glass slide with mounting media (e.g., EMS, Cat# 17987-10) and observe cells under fluorescence microscope.

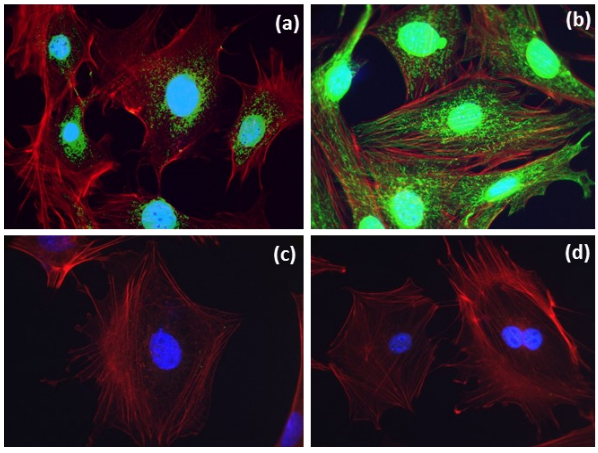

Fig 3: **Utilization of AAC02 for Immunofluorescence**. Swiss 3T3 cells, untreated (a and c) or treated (b and d) with TSA (1 μM for 6 h), were stained as described. Acetylated proteins were visualized using a green fluorescent secondary. Actin fibers were visualized using a red Rhodamine Phalloidin 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 (10ug/ml) was used to compete for AAC02 binding as an indicator of AAC02 specificity for acetyl-lysine modifications.

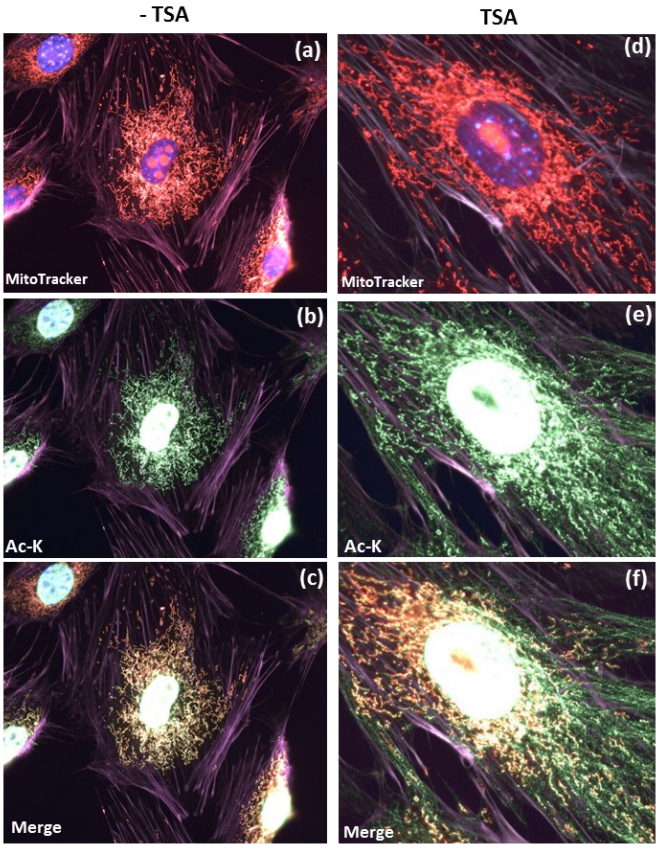

Fig 4: **AAC02 detects mitochondrial acetylation**. Swiss 3T3 cells, either untreated or treated with TSA (1 μM for 6h), were stained as described in the method. **(a and d):** Mitochondria were visualized with MitoTracker orange (Thermo Fisher). **(b and e):** Acetylated cytoplasmic and nuclear proteins 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.

Chromatin Immunoprecipitation (ChIP) Application

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

ChIP Method

1. Sheared and cross-linked chromatin from A431 cells was prepared according to published protocols (4).
2. Approximately 25 µg 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 µg/ml pepstatin/leupeptin, and aprotinin.
3. Bring the final volume to 100 µl in ChIP reaction buffer and add 1 µl 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 µl 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 µl 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 µl 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 µl of TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) and resuspend beads in 100 µl 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).

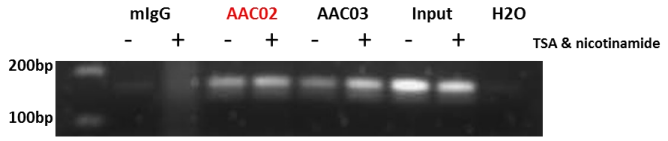

Fig 5: **Utilization of AAC02 for ChIP**. Chromatin was prepared from A431 cells, either untreated or treated with TSA (1 µM) and nicotinamide (1mM) for 6 hours. ChIP was performed as described. mIgG: mouse IgG used for ChIP control; AAC02: anti-acetyl lysine antibody used for ChIP; Input: cell lysate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

References

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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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