## Datasheet

#### Anti-Acetyl Lysine Mouse Monoclonal Antibody

Cat. # AAC03 Lot: Upon arrival, store at 4°C (desiccated) See datasheet for storage after reconstitution

Online Datasheet Contains AAC03 (V4.0) and AAC03-S (V4.0)

#### **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 which is comparable to phosphorylation in cellular prevelance (3). Antibody AAC03 detects acetyl lysine PTMs.

#### Material

Anti-acetyl lysine antibody 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 wide range of acetylated proteins (Fig. 1A), including acetylated tubulin, histones, and chemically acetylated bovine serum albumin (BSA) (Fig. 1B-C). AAC03 is 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  $4^{\circ}$ 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 100ul 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  $-20^{\circ}$ 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 100-200 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.
- 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.
- 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.
- 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.
- 10. Incubate the membrane with an appropriate dilution (e.g., 1:20,000) of anti-

Amount of material:2 x 100 µl when reconstValidated applications:WB, IF, IP, ChIPSpecies reactivity:AllHost/Isotype:Mouse/IgG1Clone:19C4B2 1	
Species reactivity: All   Host/Isotype: Mouse/IgG1	ituted
Host/Isotype: Mouse/IgG1	
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Clone: 19C4B2.1	
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- 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 at room temperature.
- 12. 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).

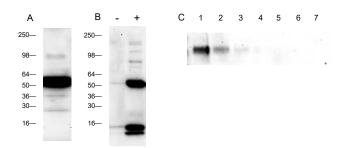


Fig 1: AAC03 was used at a 1:500 dilution following the recommended Western blot protocol (see below). Figure 1A: murine tissue extract, 30  $\mu$ g brain extract. Figure 1B: 30  $\mu$ g of Cos-7 cell lysate treated with TSA and nicotinamide (+) or untreated (-). Strongly enhanced bands at 55 and 14-16 kDa in TSA-treated lysate correspond to acetylated tubulin and histone proteins, respectively. Figure 1C: 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.

#### Immunoprecipitation (IP) Applications

Use as indicated at 20  $\mu I$  per IP reaction (1mg total lysate per IP), sufficient for approximately 10 IP assays.

#### **IP Method**

- 1. Add 20  $\mu$ l of antibody to 500  $\mu$ l of PBS pH 7.4 in a microfuge tube containing 30  $\mu$ l of packed Protein G agarose pre-equilibrated in PBS.
- 2. Gently rotate the reaction for 1 h at 4°C.
- 3. Add 500  $\mu l$  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.
- 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).
- 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.
- 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.



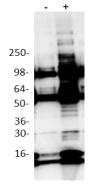


Fig 2: Cos-7cells were either treated (+) or untreated (-) with TSA (1  $\mu$ M) and nicotinamide (1mM) for 6 hours. Cell lysates were prepared in BlastR buffer and filter system and 1 mg of lysate per reaction was used for IP of acetylated proteins. 20ul of AAC03 was used per IP reaction. Western blots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

#### Immunofluorescence (IF) Applications

Use as indicated below at 1: 500 -1: 4000 dilution, sufficient for 100-800ml 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  $\mu$ 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 AAC03 (1:500) in blocking solution and incubate at room temp for 1 hr.
- 10. Wash coverslips 3 times in 0.5% Triton-X,
- Apply fluorescently-labeled anti-mouse secondary antibody at manufacturer's recommended dilution. For example, we use fluorescently-labeled goat antimouse at 1:500 dilution in blocking buffer.
- 12. Incubate at room temperature for 45 min.
- 13. Wash coverslips 3 times in PBS 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.
- Place coverslips on glass slide with mounting media (e.g., EMS, Cat# 17987-10) and observe cells under fluorescence microscope.

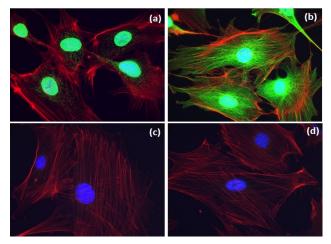


Fig 3: Utilization of AAC03 for Immunofluorescence. Swiss 3T3 cells, untreated (a and c) or treated (b and d) with TSA (1  $\mu$ M for 6 h), were stained as described. Acetylated proteins were visualized using a green fluorescent secondary. Actin fibers were visualized using a red Rhoda-mine Phalloidin and the nucleus was stained with DAPI. The acetylated microtubule network (green) 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 AAC03 binding as an indicator of AAC03 specificity for acetyl-lysine modifications.

#### Chromatin Immunoprecipitation (ChIP) Application

Use as indicated below at 1:100 dilution, sufficient for 200 ChIP assays of 100  $\mu l$  volume.

#### ChIP Method

- 1. Sheared and cross-linked chromatin from A431 cells was prepared according to published protocols (4).
- 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. Make the final volume to 100  $\mu$ l in ChIP reaction buffer and add 1  $\mu$ l of AAC03 per ChIP. NOTE: it is also recommended to run mouse IgG as a non-specific control reaction (see Ctr mIgG lanes above) and a no-antibody as input control (see Input lanes above).
- 4. Incubate on a rotator at 4°C for 1-2 h or overnight.
- 5. Add 20  $\mu l$  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 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  $\mu l$  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).
- Wash beads once with 500 µl of LiCl t uffer (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  $\mu l$  of TE burifer (10 mM Tris pH 8.0, 1 mM EDTA) and resuspend beads in 100  $\mu l$  of TE buffer.
- 12. The immunoprecipitated chromatins bound to the bead samples (and chromatins in the no-antibody control supernatant) were processed for DNA isolation and PCR analysis according to published protocols (4).

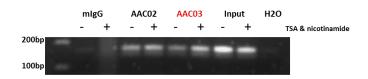


Fig 4: Utilization of AAC03 for ChIP. Chromatin was prepared from A431 cells, either untreated or treated with TSA (1  $\mu$ M) and nicotinamide (1mM) for 6 hours. ChIP was performed as described. mlgG: mouse IgG used for ChIP control; AAC02: anti-acetyl lysine antibody used for ChIP; Input: cell lysate 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.
- 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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#### Anti-Acetyl Lysine Mouse Monoclonal Antibody

Cat. # AAC03-S

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

# Form:Lyophilized powderAmount of material:1 x 25 µl when reconstitutedValidated applications:WB, IF, IP, ChIPSpecies reactivity:AllHost/Isotype:Mouse/IgG1Clone:19C4B2.1

- 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 at room temperature.
- 12. 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).

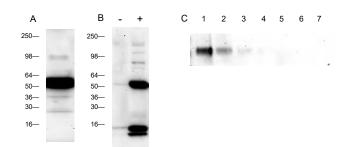


Fig 1: AAC03 was used at a 1:500 dilution following the recommended Western blot protocol (see below). Figure 1A: murine tissue extract, 30  $\mu$ g brain extract. Figure 1B: 30  $\mu$ g of Cos-7 cell lysate treated with TSA and nicotinamide (+) or untreated (-). Strongly enhanced bands at 55 and 14-16 kDa in TSA-treated lysate correspond to acetylated tubulin and histone proteins, respectively. Figure 1C: Titration of acetylated BSA lanes 1-5 contain 0.5, 0.1, 0.05, 0.01, and 0.005 ng AcBSA, lanes 6-7 contain 500 and 1000 ng non-acetylated BSA, respectively.

#### Immunoprecipitation (IP) Applications

Use as indicated at 20  $\mu$ l per IP reaction (1mg total lysate per IP), sufficient for approximately 1 IP assay.

#### IP Method

- 1. Add 20  $\mu l$  of antibody to 500  $\mu l$  of PBS pH 7.4 in a microfuge tube containing 30  $\mu l$  of packed Protein G agarose pre-equilibrated in PBS.
- 2. Gently rotate the reaction for 1 h at 4°C.
- 3. Add 500  $\mu l$  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.
- 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).
- 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.
- 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.

#### **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 which is comparable to phosphorylation in cellular prevelance (3). Antibody AAC03 detects acetyl lysine PTMs.

#### Material

Anti-acetyl lysine antibody 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 wide range of acetylated proteins (Fig. 1A), including acetylated tubulin, histones, and chemically acetylated bovine serum albumin (BSA) (Fig. 1B-C). AAC03 is 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  $4^{\circ}$ 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 25 ul 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  $-20^{\circ}$ 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.
- 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.
- 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-

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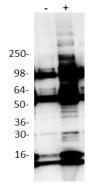


Fig 2: Cos-7cells were either treated (+) or untreated (-) with TSA (1  $\mu$ M) and nicotinamide (1mM) for 6 hours. Cell lysates were prepared in BlastR buffer and filter system and 1 mg of lysate per reaction was used for IP of acetylated proteins. 20ul of AAC03 was used per IP reaction. Western blots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

#### Immunofluorescence (IF) Applications

Use as indicated below at 1: 500 -1: 4000 dilution, sufficient for 12.5—100ml 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  $\mu$ 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 AAC03 (1:500) in blocking solution and incubate at room temp for 1 hr.
- 10. Wash coverslips 3 times in 0.5% Triton-X,
- Apply fluorescently-labeled anti-mouse secondary antibody at manufacturer's recommended dilution. For example, we use fluorescently-labeled goat antimouse at 1:500 dilution in blocking buffer.
- 12. Incubate at room temperature for 45 min.
- 13. Wash coverslips 3 times in PBS 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.
- Place coverslips on glass slide with mounting media (e.g., EMS, Cat# 17987-10) and observe cells under fluorescence microscope.

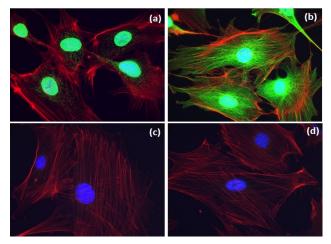


Fig 3: Utilization of AAC03 for Immunofluorescence. Swiss 3T3 cells, untreated (a and c) or treated (b and d) with TSA (1  $\mu$ M for 6 h), were stained as described. Acetylated proteins were visualized using a green fluorescent secondary. Actin fibers were visualized using a red Rhoda-mine Phalloidin and the nucleus was stained with DAPI. The acetylated microtubule network (green) 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 AAC03 binding as an indicator of AAC03 specificity for acetyl-lysine modifications.

#### Chromatin Immunoprecipitation (ChIP) Application

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

#### ChIP Method

- 1. Sheared and cross-linked chromatin from A431 cells was prepared according to published protocols (4).
- 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. Make the final volume to 100  $\mu$ l in ChIP reaction buffer and add 1  $\mu$ l of AAC03 per ChIP. NOTE: it is also recommended to run mouse IgG as a non-specific control reaction (see Ctr mIgG lanes above) and a no-antibody as input control (see Input lanes above).
- 4. Incubate on a rotator at 4°C for 1-2 h or overnight.
- 5. Add 20  $\mu l$  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 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  $\mu l$  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).
- Wash beads once with 500 µl of LiCl t uffer (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  $\mu l$  of TE burifer (10 mM Tris pH 8.0, 1 mM EDTA) and resuspend beads in 100  $\mu l$  of TE buffer.
- The immunoprecipitated chromatins bound to the bead samples (and chromatins in the no-antibody control supernatant) were processed for DNA isolation and PCR analysis according to published protocols (4).

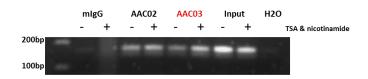


Fig 4: Utilization of AAC03 for ChIP. Chromatin was prepared from A431 cells, either untreated or treated with TSA (1  $\mu$ M) and nicotinamide (1mM) for 6 hours. ChIP was performed as described. mlgG: mouse IgG used for ChIP control; AAC02: anti-acetyl lysine antibody used for ChIP; Input: cell lysate 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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