V.1.0

Anti-Acetyl-Lysine (mAb mix) Affinity Beads

Cat. # AAC04-beads

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

Form:	Lyophilized powder
Amount of material:	4 x 500 µl when reconstituted
Validated applications:	IP
Species reactivity:	All
Host/Isotype:	Mouse/ IgG2b and IgG1
Clone:	7B5A1 and 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

AAC04 anti-acetyl lysine affinity beads are composed of a proprietary mixture of our newly developed anti-acetyl lysine antibodies, AAC02 (clone 7B5A1 / IgG2b) and AAC03 (clone 19C4B2.1 / IgG1), covalently crosslinked to protein G beads. AAC04 affinity beads enrich a more complete profile of acetylated proteins from Cos-7 cells treated with TSA and nicotinamide than either AAC02 or AAC03 affinity beads alone (Fig 1). AAC04 affinity beads are supplied as a lyophilized white powder.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized affinity beads 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 500ul of 50% glycerol and water (room temperature). Make sure the beads are completely hydrated before use. Store reconstituted affinity beads 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.

Immunoprecipitation (IP) Applications

Use as indicated at 50 µl bead slurry per IP reaction (1mg total lysate per IP), sufficient for approximately 40 IP assays.

IP Method

1. Briefly mix bead by gentle vortexing. Make sure beads are in suspension.
2. Transfer 50 µl of affinity bead slurry to 1ml of PBST in a microfuge tube with an end snipped pipet tip. Mix well and spin down beads for 1 min at 4°C and 3000 rpm.
3. Repeat PBST wash one more time.
4. 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).
5. Gently rotate the reaction at 4°C for 1-2 h or overnight if convenient.
6. Spin down beads for 1 min 4°C at 3000 rpm (approx. 960 x g).
7. Discard supernatant and wash beads with 1ml of IP wash buffer (e.g BlastR wash buffer) at 4°C.
8. Repeat wash two more times.
9. Add 30 µl of 2X **non-reducing** Laemmli buffer (125 mM Tris pH 6.8, 20% glycerol, 4% SDS, and 0.005% Bromophenol blue), mix beads well and incubate at room temp for 5 minutes.
10. Spin down beads at 3000 rpm for 1 minute at 4°C. Carefully transfer supernatant, without disturbing beads, to a new microcentrifuge tube containing 1µl of β-mercaptoethanol. Boil for 5 min prior to loading on SDS-PAGE for subsequent western blot analysis.

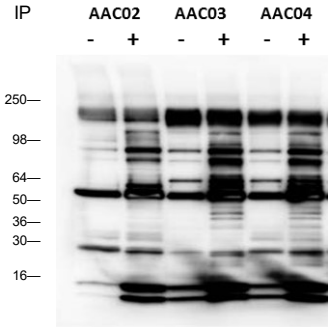

Fig 1: **Comparison of acetylated protein enrichment using various acetyl-lysine affinity beads**. AAC02, AAC03, and AAC04 acetyl lysine enrichment beads (50µl bead slurry) were used to IP acetylated proteins from Cos-7 cells either treated (+) or untreated (-) with TSA (1µM) and nicotinamide (1mM) for 6 hours. The total profile of enriched acetylated proteins were eluted and analyzed by western blot with an AAC03-HRP antibody (1:3000).

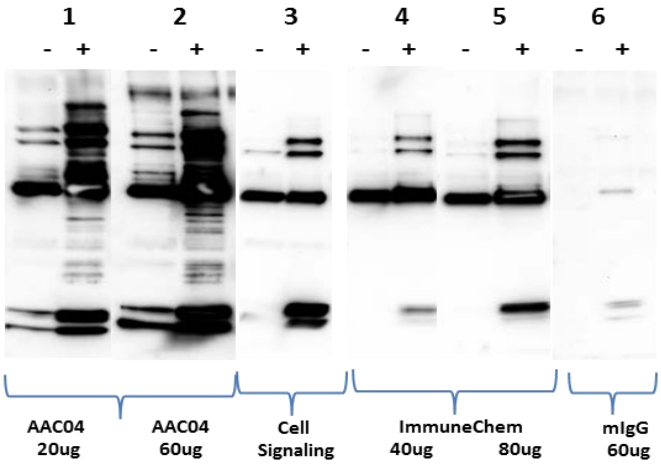

Fig 2: **Comparison of acetylated protein enrichment by AAC04 beads and established acetyl-lysine affinity reagents**. Various acetyl-lysine affinity reagents were used to IP acetylated proteins from Cos-7 cells either treated (+) or untreated (-) with TSA (1uM) and nicotinamide (1mM) for 6 hours. (1) 16.7ul of AAC04 bead slurry (20ug antibody). (2) 50ul of AAC04 bead slurry (60ug antibody). (3) Anti-acetyl lysine rabbit monoclonal mix (Cell Signaling, 1:100 per manufacturer's instruction). (4) ImmuneChem acetyl lysine affinity bead (40ug antibody). (5) ImmunChem acetyl lysine bead (80ug antibody). (6) Normal mouse IgG control bead (60ug antibody). The total profile of enriched acetylated proteins were eluted and analyzed by western blot with an AAC03-HRP antibody (1:3000). AAC04 performed exceptionally well in enriching a broad range of acetylated proteins whereas the other commercial acetyl lysine enrichment reagents only enriched the most abundance acetylated proteins (e.g. acetylated tubulin and histones).

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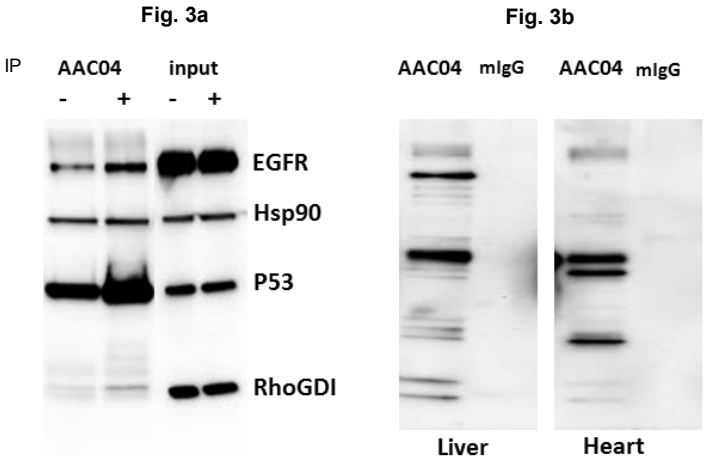

Fig 3: (A) Detection of specific acetylated target proteins. A431 cells, untreated (-) or treated (+) with 1uM TSA and 1mM nicotinamide for 6 hours, were isolated using BlastR buffer. IP was performed using AAC04 beads (60ug). Total cell lysate (Input) and immunoprecipitated samples were separated by SDS-PAGE and analyzed by western blot with antibodies against EGFR (Millipore, 1:1000), Hsp90 (Abcam, 1:20,000), P53 (Sigma, 1:2000), and RhoGDI(Millipore,1:1000).

(B) Isolation and detection of acetylated proteins from mouse tissue. Mouse tissue extracts (liver and heart) were obtained with BlastR buffer. IP was performed using AAC04 beads (60ug) or mIgG control beads (60ug) in 1mg of tissue lysate. Enriched acetylated proteins were separated by SDS-PAGE and analyzed by western blot with AAC03-HRP (1:3000).

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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