Acetyl-Lysine Affinity Beads-AAC04

Cat. # AAC04-Beads
Lot:
Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

NOW MORE VERSATILE BEAD COMPOSITION:
2 tubes each of AAC02-Beads & AAC03-Beads per pack
AAC04-Beads = 1:1 mix of AAC02-Beads:AAC03-Beads

Form:	Lyophilized powder
Amount of material:	4 x 500 µl when reconstituted
Validated applications:	IP
Species reactivity:	All
Host/Isotype:	Mouse IgG2b & 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 regulated by the opposing actions of acetyl transferases and deacetylases. Acetylation 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).

A previous formulation of AAC04-Beads comprised a pre-made 1:1 mix of two anti-acetyl mouse monoclonal antibodies (AAC02,clone 7B5A1 & AAC03, clone 19C4B2.1) covalently bound to protein G-beads. As AAC02-Beads and AAC03-Beads display overlapping but unique specificity profiles the two bead species have been separated to offer a more versatile reagent for the end user. To create AAC04-Beads simply mix AAC02-Beads & AAC03 Beads in a 1:1 ratio (25 µl of each bead slurry). For bead specific IPs use a 50 µl bead slurry of either AAC02 Bead or AAC03 Bead (see Fig 1 & Fig 2).

When examining the acetylation state of a new protein of interest (POI), it is recommended to try AAC03-Beads and AAC02-Beads separately (50 µl per IP) to determine which reagent is optimal for your POI. In the example shown in Fig 2, AAC03-Beads would be optimal for the study of EGFR and RhoGDI protein acetylation. In cases where a comprehensive acetylated protein profile is required a 1:1 mix of both beads (25 µl each per IP) (Cat # AAC04-Beads) should be considered (Fig 1).

In all cases AAC02-Beads, AAC03-Beads and AAC04-Beads are able to outperform other commercially available acetylated protein enrichment beads (Fig 3).

Material

AAC02-Beads and AAC03-Beads are composed of an anti-acetyl lysine mouse IgG2b (clone 7B5A1) and IgG1 antibody (clone 19C4B2.1) respectively. Each antibody is covalently crosslinked to protein G beads. The beads are supplied as a white lyophilized 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 500 µl of room temperature 50% glycerol (1 part glycerol to 1 part sterile water). Allow beads to re-hydrate for 30 minutes at room temperature. 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 bead stock.

Fig 1: Comparison of acetylated protein enrichment using various acetyl-lysine affinity bead

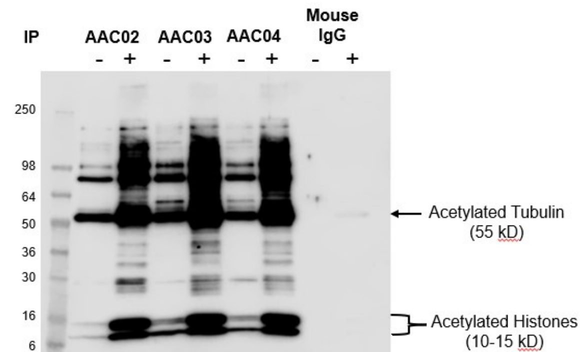
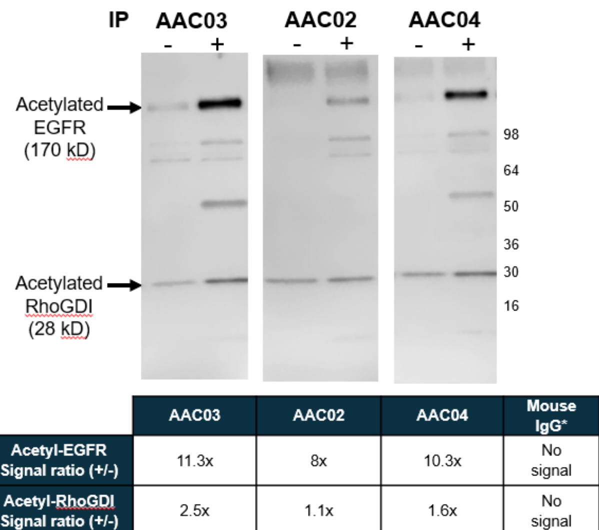


Fig 1 Legend:
AAC02-Beads, AAC03-Beads, and AAC04-Beads (50µl bead slurry) were used to IP acetylated proteins from Cos-7 cells either treated (+) or untreated (-) with deacetylase inhibitors [TSA (1µM) and nicotinamide (1mM)] for 6 hours. The total profile of enriched acetylated proteins were eluted and analyzed by western blot with an AAC03-HRP antibody (1:3000). Mouse IgG beads are used as a control for non-specific binding (Cat # CIG02). Each IP assay utilized 1 mg of Cos-7 lysate.

Fig 2: IP Efficiency for Acetylated EGFR & RhoGDI proteins using Acetyl Affinity Beads



	AAC03	AAC02	AAC04	Mouse IgG*
Acetyl-EGFR Signal ratio (+/-)	11.3x	8x	10.3x	No signal
Acetyl-RhoGDI Signal ratio (+/-)	2.5x	1.1x	1.6x	No signal

Fig 2 Legend: AAC03-Beads & AAC02-Beads (50µl bead slurry) and a 1:1 mix (25 µl each) AAC04-Beads, were used to IP acetylated proteins from Cos-7 cells either treated (+) or untreated (-) with deacetylase inhibitors, [TSA (1µM) and nicotinamide (1mM)], for 6 hours. Western blot analysis using anti EGFR and anti -RhoGDI antibodies was performed and signals were quantitated using LiCor Empiria software. IP assays were also carried out and signals quantitated for mouse IgG control beads (blots not shown). Each IP used 1 mg of lysate.

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Fig 3: Comparison of acetylated protein enrichment by AAC04-Beads and established acetyl-lysine affinity reagents

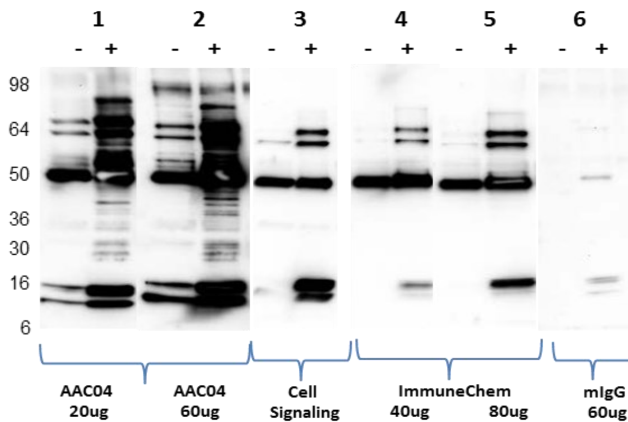

Fig. 3 Legend:
Various acetyl-lysine affinity reagents were used to IP acetylated proteins from Cos-7 cells either treated (+) or untreated (-) with TSA (1µM) and nicotinamide (1mM) for 6 hours. (1) 16.7ul of AAC04 Bead slurry (10µg AAC02-Beads & 10µg AAC03-Beads). (2) 50ul of AAC04 Bead slurry (30µg AAC02-Beads & 30µg AAC03-Beads). (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 (80µg antibody). (6) Normal mouse IgG control bead (60µg antibody). The total profile of enriched acetylated proteins were eluted and analyzed by western blot with an AAC03-HRP antibody (1:3000). AAC04-Beads 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 [55kD]and histones [10-15 kD]).

Immunoprecipitation (IP) Applications

Use as indicated at 50 µl bead slurry per IP reaction for AAC02-Beads or AAC03-Beads alone or 25 µl each of AAC02-Beads & AAC03-Beads for an AAC04 Bead IP. Sufficient reagents for approximately 40 IP assays for AAC04-Beads or 20 assays each for AAC02-Beads & AAC03-Beads.

IP Method

1. Remove beads from –20°C and warm to 4°C on ice.
2. Make sure beads are in suspension by mixing gently end over end by hand.
3. Transfer 50 µl of affinity bead slurry for an AAC02-Beads or AAC03-Beads per IP or 25 µl each of AAC02/AAC03 for an AAC04 Bead IP 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.
4. Repeat PBST wash one more time.
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 that contains deacetylase inhibitors, NOTE 1.
6. Gently rotate the reaction at 4°C for 2 h (optimal) or overnight if convenient.
7. Spin down beads 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 (optimal) or RIPA buffer: see NOTE 1) at 4°C.
9. Repeat wash two more times.
10. 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 using either a wide bore pipette or by flicking the tube and incubate at room temp for 5 minutes.
11. 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.

NOTE 1: BlastR lysis buffer was used to generate the data shown in this data sheet and is highly recommended for optimal sensitivity. RIPA buffer, see recipe below, and other common lysis buffers can be used but they are inferior to the BlastR system (see White paper for BlastR system validations: Comparison of the BlastR Rapid Lysate prep kit with conventional lysis buffers: https://www.cytoskeleton.com/about-signal-seeker-validation-data). Deacetylase inhibitors should be added to lysis buffer before use.

RIPA IP Buffer Recipe (100 ml)

Component	Volume	Final Concentration
1M Tris pH 7.6	2.5 ml	25 mM
1M NaCl	15 ml	150 mM
NP-40	1 ml	1%
10% sodium deoxycholate	10 ml	1%
10% SDS	1 ml	0.1%
Water	73 ml	na

References

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