V.2.0

Acetyl-Lysine Affinity Beads-AAC02

Cat. # AAC02-Beads
Lot:
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
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 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).

Cytoskeleton offers two types of Acetyl-Lysine Affinity Beads, Cat # AAC02-Beads and Cat # AAC03-Beads. Both reagents are comprised of mouse anti-acetyl-lysine antibodies that are covalently linked to protein G beads and both enrich a broad range of acetylated proteins (Fig. 1). AAC02-Beads and AAC03-Beads can be combined to give a more extensive acetylated protein enrichment profile (Cat # AAC04-Beads is a 1:1 combination of AAC02-Beads:AAC03-Beads).

AAC02-Beads has an overlapping but unique specificity profile when compared to AAC03-Beads and may outperform AAC03-Beads when examining a specific target protein as has been found in the case of PDHE1 (Fig. 2). In the presence of hydrogen peroxide AAC02 Bead IPs show a 2.6 fold de-acetylation of PDHE1 while AAC03 Bead shows a 1.5 fold de-acetylation and 8-9 fold weaker signal (Fig. 2). The specificity of the beads for acetylated PDHE1 is shown by the lack of signal from mouse IgG control beads (Fig. 2). A potential mechanism for deacetylation in response to hydrogen peroxide treatment is the upregulation of the deacetylase SIRT3 (5).

Similarly, AAC03-Beads may outperform AAC02-Beads for specific target proteins as is the case for epidermal growth factor receptor (EGFR) and RhoGDI proteins (Fig 3). In the presence of deacetylase inhibitors AAC03 Bead IPs show a 4.0 fold and 2.8 fold enhancement in acetylated EGFR and RhoGDI signal respectively while AAC02-Beads shows a weaker 1.4 fold and 1.6 fold enhancement respectively.

In all cases AAC02-Beads, AAC03-Beads and AAC04-Beads are able to outperform other commercially available acetylated protein enrichment beads (Fig. 4).

When examining the acetylation state of a new protein of interest (POI), it is recommended to try AAC02-Beads and AAC03-Beads separately to determine which reagent is optimal for your POI. In cases where a comprehensive acetylated protein profile is required the use of both beads (Cat # AAC04-Beads) should be considered.

Material
AAC02-Beads are composed of an anti-acetyl lysine mouse IgG2b antibody (clone 7B5A1) that is covalently crosslinked to protein G beads. The beads are supplied as a white lyophilized powder. AAC02-Beads immunoprecipitate a comprehensive set of acetylated proteins (Fig 1-4).

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 beads

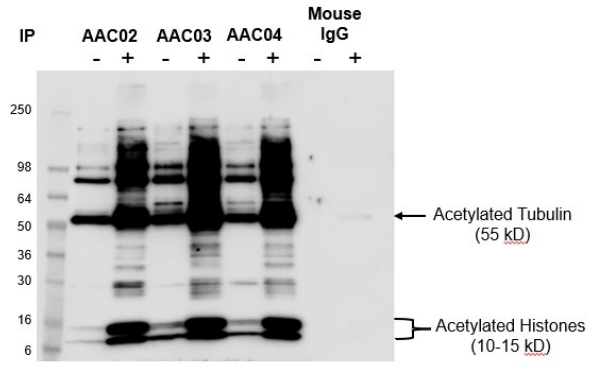
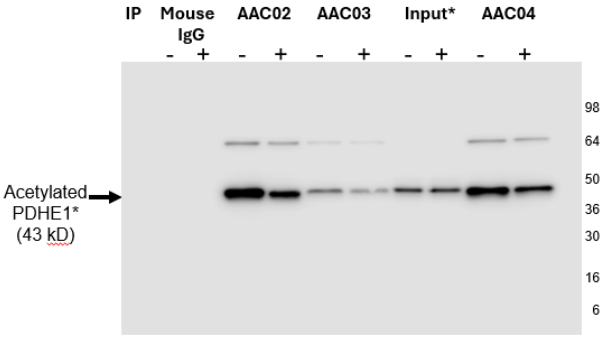


Fig 1 Legend: AAC02-Beads, AAC03-Beads, and AAC04-Beads (50µl bead slurry) were used to IP acetylated proteins from Cos-7 cells either treated (+) or untreated (-) with deacetylase inhibitors [TSA (1µM) and nicotinamide (1mM)] for 6 hours. The total profile of enriched acetylated proteins were eluted 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 utilizes 1 mg Cos-7 lysate.

Fig 2: AAC02 Enhanced IP Efficiency for Acetylated PDHE1



	AAC02	AAC03	Input	AAC04
Acetyl-PDHE1 Fold De-acetylation (-/+)	2.6X	1.5X	0.9X	1.8X

*Input signal is from total lysate signal, both acetylated and non-acetylated PDHE1 and does not represent an IP

Fig 2 Legend: AAC02-Beads & AAC03-Beads (50µl bead slurry) were used to IP acetylated proteins from A431 cell lysates either treated (+) or untreated (-) with hydrogen peroxide (100 µM) for 2 hours. Each IP used 1 mg of lysate. Western blot analysis using anti PDHE1 antibody was performed and signals were quantitated using LiCor Empiria software (Table).
Mouse IgG control beads (Cat #CIG02) and AAC04-Beads (25 µl AAC02-Beads & 25 µl AAC03-Beads) were also used in IP assays (1 mg lysate per IP). Input lanes represent 2% of IP input lysate (20 µg) from treated (+) or untreated (-) lysate. Input signal represents total PDHE1.

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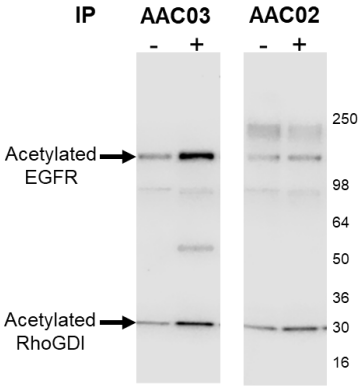
Fig 3: AAC03 Enhanced IP Efficiency for Acetylated EGFR & RhoGDI



	AAC03	AAC02
Acetyl-EGFR Signal ratio (+/-)	4.0x	1.4x
Acetyl-RhoGDI Signal ratio (+/-)	2.8x	1.6x

Fig 3 Legend: AAC03-Beads & AAC02-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. Western blot analysis using anti EGFR and anti-RhoGDI antibodies was performed and signals were quantitated using LiCor Empiria software. Each IP assay utilized 1 mg of Cos-7 lysate.

Fig 4: Comparison of acetylated protein enrichment by AAC04-Beads and established acetyl-lysine affinity reagents

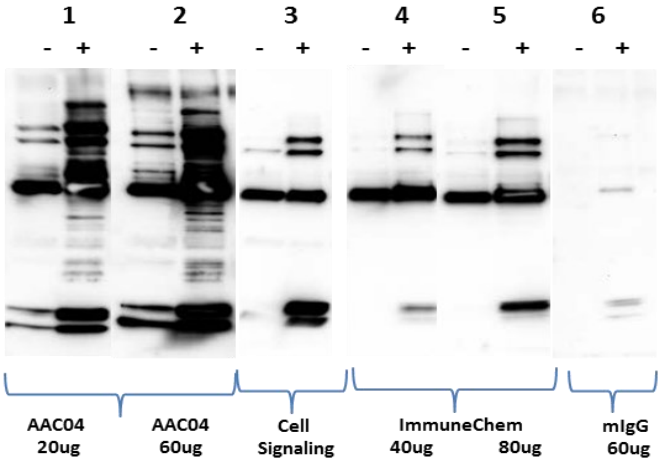


Fig 4 Legend: Various acetyl-lysine affinity reagents were used to IP acetylated proteins from Cos-7 cells either treated (+) or untreated (-) with TSA (1uM) and nicotinamide (1mM) for 6 hours. (1) 16.7ul of AAC04 Bead slurry (20ug antibody). (2) 50ul of AAC04 Bead slurry (60ug antibody). (3) Anti-acetyl 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).

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. 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 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, 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).

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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