V 2.0

Chemically Acetylated Bovine Serum Albumin (BSA)
Cat. # AACX1

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

Background Information

Post-translational acetylation of proteins occurs on the epsilon group of lysine residues. Lysine acetylation, *in vivo*, is carried out by a group of enzymes termed HATs (histone acetyl transferases) or, more generally, LATs (lysine acetyl transferases) (1). Lysines can also be chemically acetylated *in vitro* by acetic anhydride under basic conditions (2). Chemically acetylated proteins are useful substrates for determination of acetyl lysine antibody sensitivity and specificity.

Material

Lysine residues of bovine serum albumin (BSA) have been chemically acetylated by acetic anhydride under basic conditions. A major Ac-BSA band runs at approximately 66 KDa and Ac-BSA oligos run at approximately 120-260 KDa. NOTE: Ac-BSA oligomerization is not caused by the acetylation reaction as these species are also present in unmodified BSA as seen in a Coomassie stain of unmodified BSA protein (data not shown). Acetylated BSA is supplied as a lyophilized powder.

Storage and Reconstitution

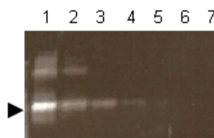
Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for up to 12 months.

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 water to give a 1mg/ml protein stock. Reconstituted protein can be aliquoted into experiment sized volumes and stored at –20°C. Avoid freeze/thaw cycles.

Applications

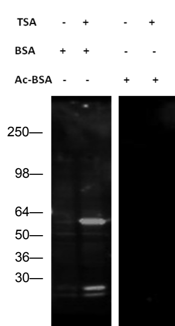
Can be used as a positive control for anti-acetyl antibody specificity and sensitivity (Fig.1) and as a competitive inhibitor for antibody binding to acetyl lysine targets (Fig. 2).

Figure 1: Titration of Ac-BSA to determine antibody specificity and sensitivity



Legend: Lanes 1-5, 0.5, 0.1, 0.05, 0.01 and 0.005 ng Ac-BSA respectively. Lanes 6 & 7, 500 and 1000 ng of non-acetylated BSA. Western blots of Ac-BSA and unmodified BSA were analysed using AAC01 anti-acetyl antibody. The 1000 ng of non-acetylated BSA did not produce a signal while Ac-BSA was detected down to 0.005 ng. The major BSA band runs at approximately 66KD (arrowhead) with BSA oligomers running between approximately 120-260 KDa.

Figure 2: Ac-BSA competition for antibody binding to acetyl lysine targets



Legend: Lysates were made from A431 cells that were treated (+) or untreated (-) with the HDAC inhibitor trichostatin A (TSA). Each lane represents 20 µg of cell lysate. Lysates were run on SDS-PAGE and transferred to PVDF membranes. Membranes were probed with anti-acetyl lysine antibody (Cat# AAC01) at 1:500 dilution in TBST in the presence of 10 µg/ml BSA or 10 µg/ml of Ac-BSA. The Ac-BSA eliminates all acetyl lysine signal on the membrane. Prominent bands in the TSA treated lanes represent acetyl tubulin (55 KDa) and acetyl histones (14-16 KDa).

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Western Blot Method:

1. Run protein samples and control samples in SDS-PAGE.
2. 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.
3. Transfer the protein to a PVDF membrane for 60 min at 75 V.
4. Wash the membrane with TBST for 10 min. with constatnt agitation (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
5. Air dry the membrane at room temperature for 30 min.
6. Rehydrate the membrane in TBST for 30 min. at room temperature.
7. The membrane may be left in TBST overnight at 4° C if convenient.
8. Block the membrane surface with 5% nonfat-dry milk in TBST for 30 min at room temperature with constant agitation.
9. Incubate the membrane with a 1:500-1:1000 dilution of anti-acetyl lysine antibody, diluted in TBST, for 1-2 h at room temperature or overnight at 4°C with constant agitation. If Ac-BSA is to be used as a competitor, then include this at 10 µg/ml.
10. Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
11. 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/0.5% non-fat milk for 60 min at room temperature with constant agitation.
12. Wash the membrane 6 times in TBST for 10 min each with constant agitation.
13. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

NOTE: This Ab has some cross reactivity with the dyes used for pre-stained molecular weight markers. This does not affect protein specificity for acetyl groups (see Fig. 1 & 2). Also, some molecular weight marker proteins are acetylated, particularly commonly used metabolic enzymes such as glutamic dehydrogenase (m.wt. approx. 55kDa).

References

1. Yang X-J. 2004. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res*. **32**, 959-976.
2. Komatsu Y. et al. 2003. Four different clones of mouse anti-acetyllysine monoclonal antibodies having different recognition properties share a common immunoglobulin framework structure. *J. Immunol. Meth*ods **272**, 161-175.

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