Tricostatin A (TSA) treated and untreated cell lysates
Cat. # AACX2

V 1.0

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

Background Information

The post-translational acetylation of lysine residues is regulated through the opposing activities of acetylase and deacetylase enzymes (1). As acetylation/deacetylation is often a highly dynamic process, reagents that can fix proteins in their acetylated state are useful tools when studying the acetylome. Deacetylase inhibitors, such as tricostatin A (TSA), allow the accumulation of acetylated proteins and aid in the identification and characterization of proteins/pathways regulated by acetylation (2). Tricostatin A-treated cell lysates are also useful as positive controls for anti-acetyl lysine antibodies.

Material

A431 cells were grown in a CO_2 incubator at 37°C to approximately 70% confluency in DMEM media supplemented with 10% fetal bovine serum. Cells were subsequently treated with 0.66 µM TSA in DMSO (TSA treated cells) or with DMSO only (untreated cells) and returned to the incubator for 5h. Cell lysates were subsequently harvested. TSA treated and untreated lysates are supplied as lyophilized powder.

Storage and Reconstitution

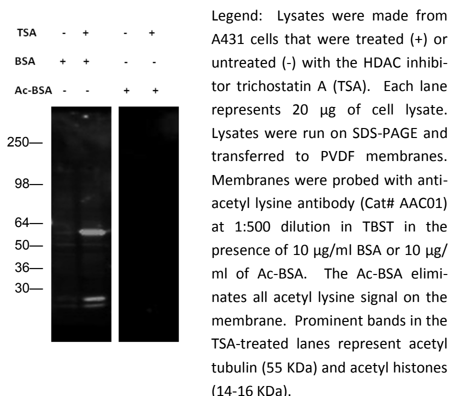
Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for up to 12 months.

For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube. Reconstitute each tube in 100 µl of 1X Laemmli buffer (63 mM Tris pH6.8, 10% glycerol, 2% SDS, 0.003% Bromophenol blue, 2.5% beta-mercaptoethanol) to give a 1 mg/ml protein stock. Reconstituted protein can be aliquoted into experiment-sized volumes (20 µl is recommended per lane of an SDS-PAGE minigel) and placed at –20°C for storage. Avoid repeated freeze/thaw cycles.

Applications

Recommended as a positive control for anti-acetyl lysine antibodies.

Figure 1: Western blot of TSA-treated and untreated lysates probed with an anti-acetyl lysine MAb



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).

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.

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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 5 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. Norris K. et al. 2009. Acetylation goes global: The emergence of acetylation biology. *Sci. Signal*. **2 (97)**, pe76.

2. Kim S-H. et al. 2010. Trichostatin A enhances acetylation as well as protein stability of Era through induction of p300 protein. *Breast Cancer Res*. **12**, R22.

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