K-AcylStat Panel, SNAP-ChIP™ spike-in

Catalog Numbers A47358, A47359

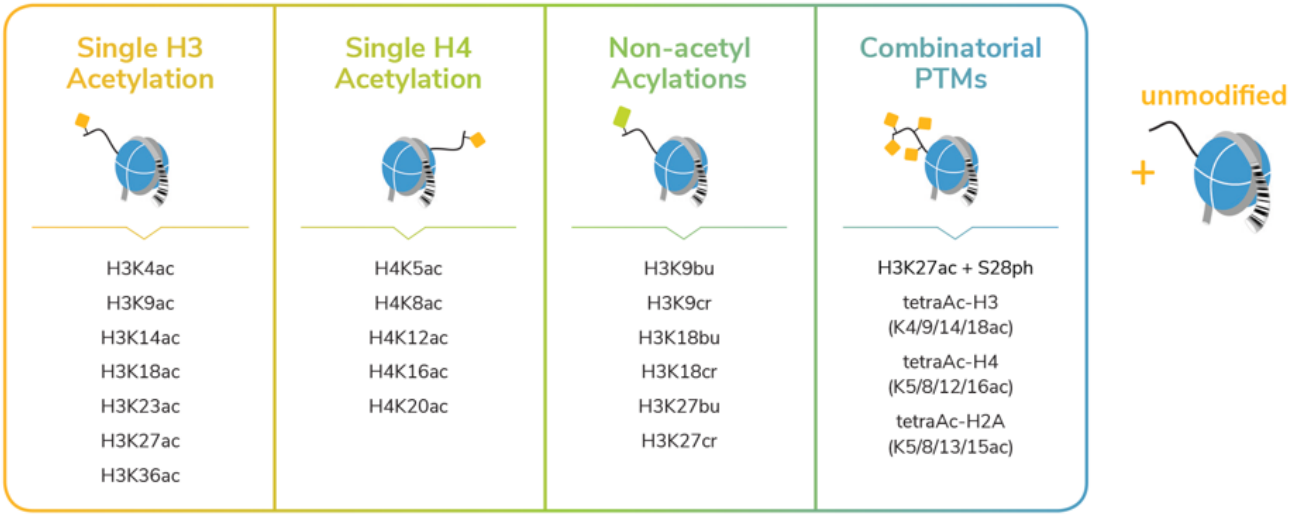
Pub. No. MAN0019260 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product Description

SNAP-ChIP™ spike-in, a proprietary method developed by EpiCypher™, is a panel of distinctly modified mononucleosomes assembled from recombinant human histones expressed in *E. coli* (two each of histones H2A, H2B, H3, and H4; accession numbers: H2A-P04908; H2B-O60814; H3.1-P68431 or H3.2-Q71DI3*; H4-P62805). Each histone is wrapped by 147 base pairs of barcoded Widom 601 positioning sequence DNA. The mononucleosomes constitute a pool of 1 unmodified histone plus 22 modified histones (see Table 1). The modified histones are either H2A, H3, or H4 histones with post-translational modifications (PTMs), created by a proprietary semi-synthetic method. Each distinctly modified nucleosome is distinguishable by a unique sequence of DNA (the "barcode") at the 3' end that can be deciphered by qPCR or next-generation sequencing.

** Histone H3.2 contains a Cys to Ala substitution at position 110.*



Contents and storage

Stable for one year at -20°C from date of receipt.

Formulation

Purified recombinant mononucleosomes containing:

- A mixture of 23 histones in 10 mM sodium cacodylate, pH 7.5, 100 mM NaCl, 1 mM EDTA, 50% glycerol (w/v), 1x Protease Inhibitor cocktail, 100 µg/mL BSA, and 10 mM β-mercaptoethanol.
- Average molarity = 0.6 nM.
- MW = ~199382.1 Da (average MW of all 23 nucleosomes).

Application notes

K-AcylStat Panel, SNAP-ChIP™ spike-in barcoded nucleosome standards are highly purified recombinant mononucleosomes and are suitable for use as spike-in controls for ChIP reactions, for antibody specificity testing or for effector protein binding experiments. For more information, see *SNAP-ChIP Panels User Guide* (Pub. No. MAN0019284).

Expected results

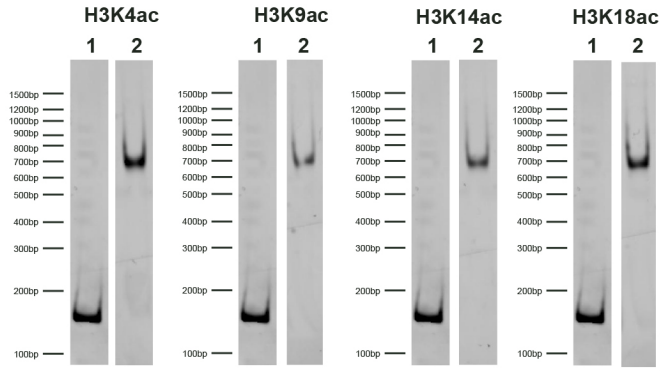


Figure 1 DNA gel data

Representative images for SNAP-ChIP™ K-AcylStats resolved by native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free 147 bp DNA used in nucleosome assembly (100 ng). **Lane 2:** Intact nucleosomes (200 ng). Comparable experiments were performed for the entire K-AcylStat panel.

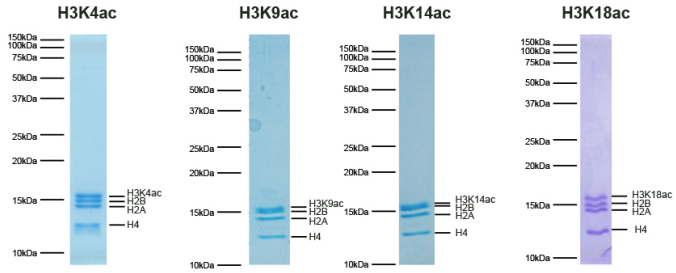


Figure 2 Protein gel data

Representative Coomassie stained PAGE gel of SNAP-ChIP™ K-AcylStat (2 µg each) to demonstrate the purity of the histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3 and H4) are indicated. Comparable experiments were performed for the entire K-AcylStat panel.

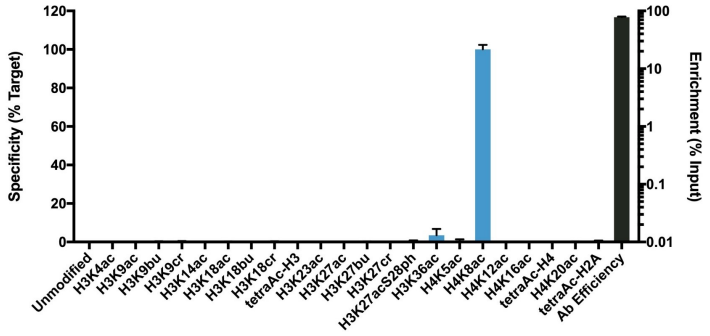


Figure 3 Representative SNAP-ChIP data

K-AcylStat Panel, SNAP-ChIP™ spike-in (Cat. No. A47358) was used to analyze the performance of recombinant monoclonal H4K8ac antibody (Product # 701796) in ChIP. SNAP-ChIP™ panels consist of a pool of DNA-barcoded recombinant nucleosomes harboring unique histone post-translational modifications (PTMs, on- and off-target) that are spiked-in to a ChIP reaction early in the workflow. The K-AcylStat panel includes an unmodified control plus nucleosomes with single acylations (acetylation, butyrylation, or crotonylation) on H3 (K4ac, K9ac/bu/cr, K14ac, K18ac/bu/cr, K23ac, K27ac/bu/cr, K36ac), H4 (K5ac, K8ac, K12ac, K16ac, K20ac) as well as combinatorial PTMs including H3K27ac+S28ph, tetraAc-H3 (K4,9,14,18ac), tetraAc-H4 (K5,8,12,16ac), and tetraAc-H2A (K5,8,13,15ac). Recovery of each unique DNA-barcoded nucleosome is quantified to determine how much of each PTM is immunoprecipitated in the ChIP reaction. H4K8ac was tested in native ChIP with 3 µg K-652 cell chromatin and 3 µg antibody. Specificity (left Y-axis) was determined by quantitative real-time PCR (qPCR) to each modified nucleosome in the SNAP-ChIP OncoStat panel (X-axis). Black bar represents antibody efficiency (right Y-axis; log scale) and indicates percentage of the barcoded nucleosome target immunoprecipitated relative to Input. All bars represent mean ± SEM.

References using this product

SNAP-ChIP is adapted from Grzybowski AT et al. (2015) Mol Cell 58: 886-889.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.