

K-AcylStat Panel

Cat. No. NUC-064 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	A panel of distinctly modified mononucleosomes assembled from recombinant human histones expressed in E. coli.
Source	E.coli
Description	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) wrapped by 147 base pairs of barcoded Widom 601 positioning sequence DNA. The panel is comprised of a pool of 1 unmodified plus 22 histone H2A, H3, or H4 post-translational modifications (PTMs, created by a proprietary semi-synthetic method): H3K4ac, H3K9ac, H3K14ac, H3K18ac, H3K23ac, H3K27ac, H3K36ac, H4K5ac, H4K8ac, H4K12ac, H4K16ac, H4K20ac, H3K9bu, H3K9cr, H3K18bu, H3K18cr, H3K27bu, H3K27cr, H3K27acS28phos, H3K4,9,14,18ac, H4K5,8,12,16ac, H2AK5,8,13,15ac. Each distinctly modified nucleosome is distinguishable by a unique sequence of DNA ("barcode") at the 3' end that can be deciphered by qPCR or next-generation sequencing. Each of the 23 nucleosomes in the pool is wrapped by 2 distinct DNA species, each containing a distinct barcode ("A" and "B", see SNAP-ChIP Manual) allowing for an internal technical replicate.
Form	PPurified recombinant mononucleosomes, containing a mixture of 23 (1 unmodified plus 22 unique) H2A, H3, and H4 PTMs in 10 mM sodium cacodylate, pH 7.5, 100 mM NaCl, 1 mM EDTA, 50% glycerol (w/v), 1x Protease Inhibitor cocktail, 100 g/mL BSA, 10 mM β-mercaptoethanol. Average molarity = 0.6 nM.

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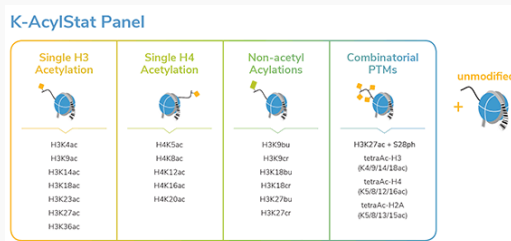
Molecular Mass ~199382.1 Da (average MW of all 23 nucleosomes)

Applications

SNAP-ChIP K-AcylStat 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. See product manual for more information. SNAP-ChIP can easily be introduced into existing ChIP and ChIP-seq workflows for experiment normalization and monitoring of technical variability.

Storage

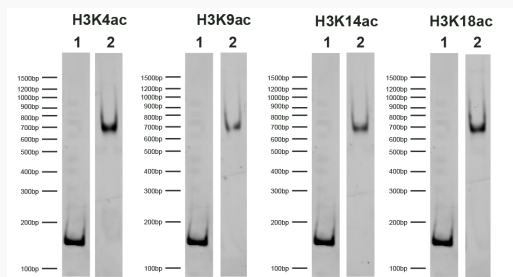
Stable for six months at -20°C from date of receipt.



SNAP-ChIP OncoStat Panel

Schematic representation of nucleosomes comprising the K-AcylStat Panel: H3K4ac, H3K9ac, H3K14ac, H3K18ac, H3K23ac, H3K27ac, H3K36ac, H4K5ac, H4K8ac, H4K12ac, H4K16ac, H4K20ac, H3K9bu, H3K9cr, H3K18bu, H3K18cr, H3K27bu, H3K27cr, H3K27acS28phos, H3K4,9,14,18ac, H4K5,8,12,16ac and unmodified.

DNA Gel Data

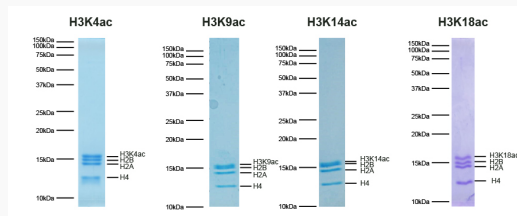


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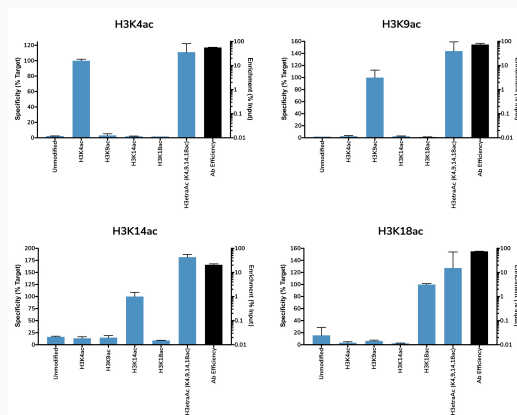
Representative images for SNAP-ChIP K-AcylStats resolved by native PAGE and stained with ethidium bromide to visualize DNA. Lane 1: Free 147bp DNA used in nucleosome assembly (100 ng). Lane 2: Intact nucleosomes (200 ng). Comparable experiments were performed for the entire K-AcylStat Panel.



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.

ChIP Data



Representative chromatin immunoprecipitation (ChIP) data using commercially available ChIP-grade antibodies targeting the indicated PTMs. The antibodies were



assayed in a native ChIP experiment with 3 μ g antibody added to 3 μ g K-562 chromatin with the K-AcylStat Panel spiked-in prior to micrococcal nuclease digestion. Quantitative real-time PCR (qPCR) was used to measure recovery of duplicate DNA barcodes corresponding to each uniquely modified nucleosome in the panel (blue bars, X-axis). The black bars map to the log scale on the right y-axis and indicate the percentage of target immunoprecipitated relative to the input (a measure of the antibody efficiency). In each case, the SNAP-ChIP spike-in confirmed that the antibodies recovered the expected histone PTM with high efficiency and specificity. Comparable experiments were performed for the entire K-AcylStat Panel.