

SNAP-ChIP[®] K-AcylStat[™] Panel

Catalog No	19-3001	Pack Size	20 μ L
Lot No	23011003-05	Concentration	0.6 nM per nucleosome

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 mononucleosomes constitute 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. *Note: Histone H3.2 contains a Cys to Ala substitution at position 110.

TECHNICAL INFORMATION

Storage	Stable for six months at -20°C from date of receipt.
Formulation	Purified 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 μ g/mL BSA, 10 mM β -mercaptoethanol. Average molarity = 0.6 nM. MW = ~199382.1 Da (average MW of all 23 nucleosomes).

APPLICATION NOTES

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.

REFERENCES

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

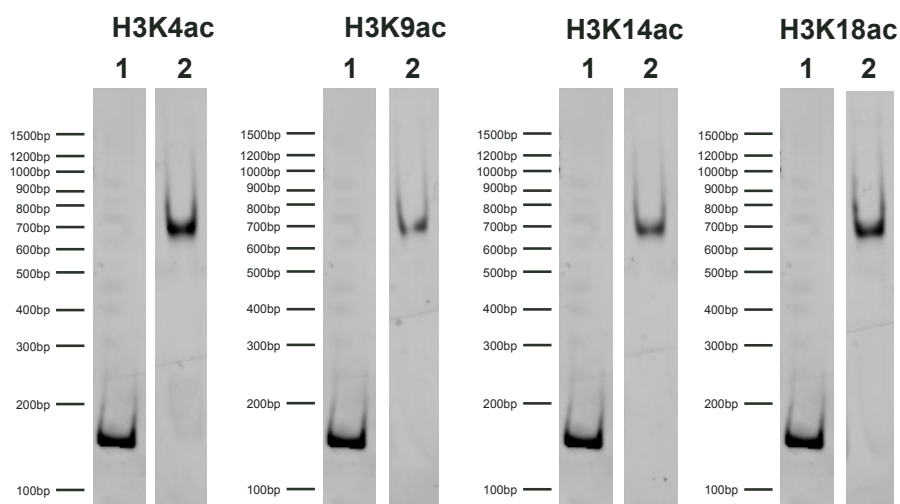


FIGURE 1 DNA gel data. Representative images for SNAP-ChIP K-AcylStat resolved by native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free 147bp DNA used in nucleosome assembly (100 ng). **Lane 2:** Intact nucleosomes (200 ng). Comparable experiments were performed for the entire K-AcylStat Panel. Email techsupport@epicypher.com for more information.

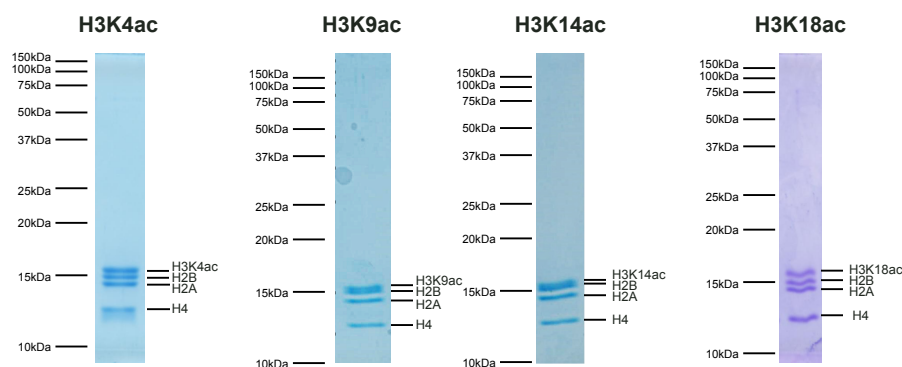


FIGURE 2 Protein gel data. Representative Coomassie stained PAGE gel of SNAP-ChIP K-AcylStat (2 µg each) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3, and H4) are indicated. Comparable experiments were performed for the entire K-AcylStat Panel. For more information email techsupport@epicypher.com.

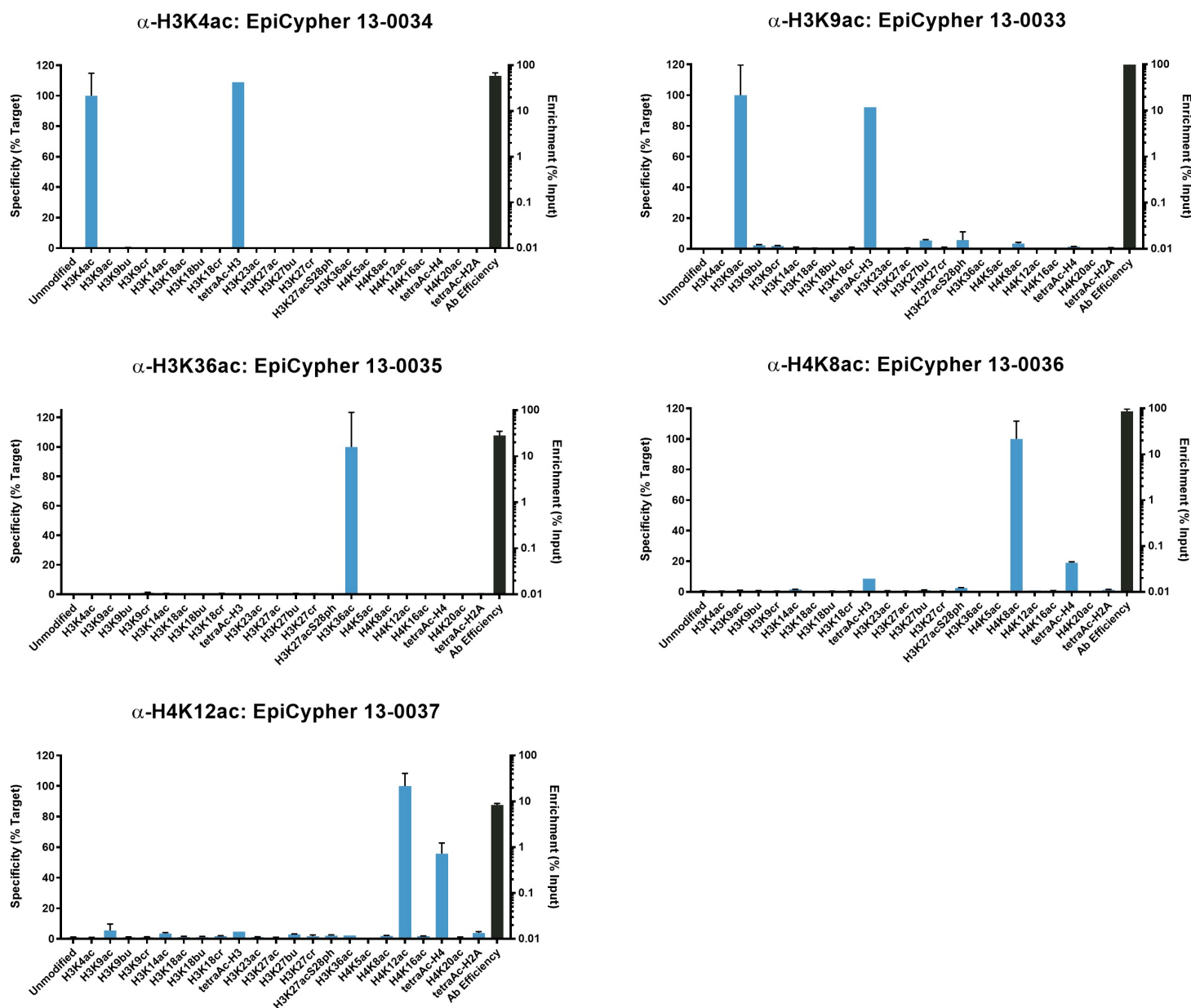


FIGURE 3 ChIP data. Representative chromatin immunoprecipitation (ChIP) data using the indicated EpiCypher SNAP-ChIP Certified antibodies targeting the indicated PTMs. The antibodies were assayed in a native ChIP experiment with 3 μ g antibody added to 3 μ g K-562 chromatin with the K-AcylStat Panel 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. For more information, email techsupport@epicypher.com.