K-MetStat Panel, SNAP-ChIP™ spike-in

Catalog Numbers A47356, A47357

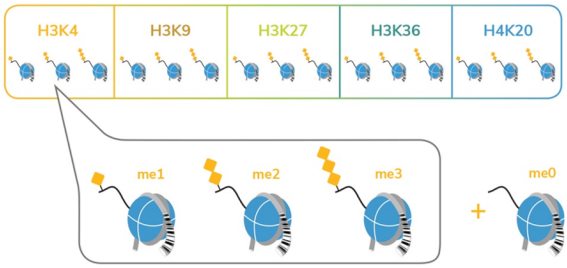
Pub. No. MAN0019258 Rev. A.0

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 15 modified histones (see Table 1). The modified histones are either 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 16 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 16 nucleosomes).

Application notes

K-MetStat 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

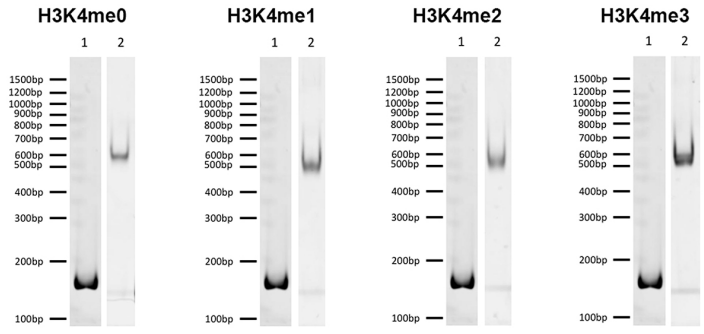


Figure 1 DNA gel data

Representative images for K-MetStat Panel, SNAP-ChIP™ spike-in nucleosomes resolved by native PAGE and stained with ethidium bromide to confirm intact nucleosome assembly with minimal free DNA. **H3K4me0** = unmodified. **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-MetStat panel.

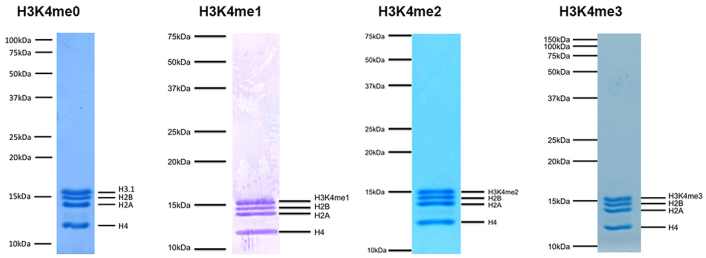


Figure 2 Protein gel data

Representative Coomassie stained PAGE gel of K-MetStat Panel, SNAP-ChIP™ spike-in nucleosomes (2 µg each) to demonstrate the purity of the histones in the preparation. **H3K4me0** = unmodified. 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-MetStat panel.

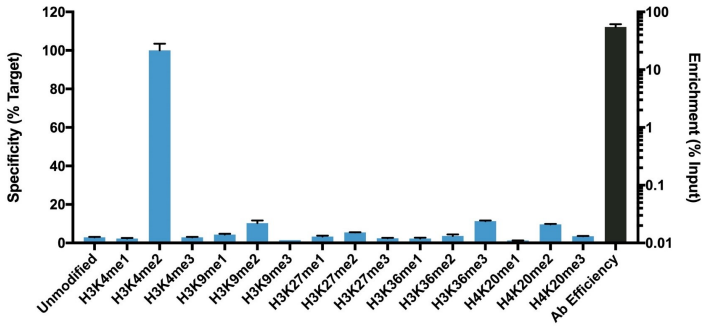


Figure 3 Representative SNAP-ChIP data

K-MetStat Panel, SNAP-ChIP™ spike-in (Cat. No. A47356) was used to analyze the performance of recombinant monoclonal H3K4me2 antibody (Product # 701764) 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-MetStat panel includes unmethylated, mono, di, and tri-methyl forms of H3K4, H3K9, H3K27, H3K36 and H4K20 nucleosomes. Recovery of each unique DNA-barcoded nucleosome is quantified to determine how much of each PTM is immunoprecipitated in the ChIP reaction. H3K4me2 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 K-MetStat Panel, SNAP-ChIP™ spike-in (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

1. SNAP-ChIP is adapted from Grzybowski AT et al. (2015) Mol Cell 58: 886-889.
2. Shah RN *et al* (2018) *Mol Cell*72:162-177
3. Janssen A *et al* (2019) *Genes Dev*33:103-115
4. Lam KG *et al* (2019) *Nat Commun* 10: 3821

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