SNAP-ChIP® K-MetStat™ Panel

Catalog No 19-1100
Lot No 22145005-06
Pack Size 200 µL

Product 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 15 histone H3 or H4 post-translational modifications (PTMs, created by a proprietary semi-synthetic method): H3K4, K9, K27, and H4K20 with me1, me2, or me3. 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 16 nucleosomes in the pool is wrapped by 2 distinct DNA species, each containing a unique barcode (" A" and "B", see SNAP-ChIP Manual) allowing for an internal technical replicate. * *Histone H3.2 contains a Cys to Ala substitution at position 110.*

Formulation:

Purified recombinant mononucleosomes, containing a mixture of 16 (1 unmodified plus 15 unique) 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 µg/mL BSA, 10 mM β-mercaptoethanol. Average molarity = 0.6 nM.
MW = ~199382.1 Da (average MW of all 16 nucleosomes).

Storage and Stability:

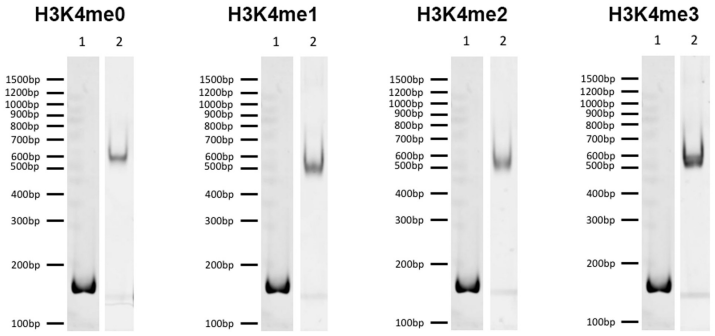
Stable for six months at -20°C from date of receipt.

Application Notes:

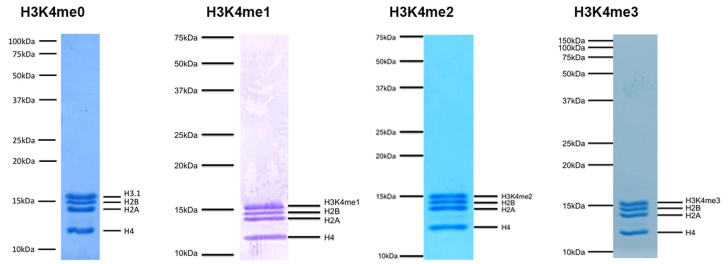
SNAP-ChIP K-MetStat Panel consists of a pool of highly purified recombinant mononucleosomes and is suitable for use as spike-in controls for ChIP reactions, for antibody specificity testing or for effector protein binding experiments. See manual for more information.

References:

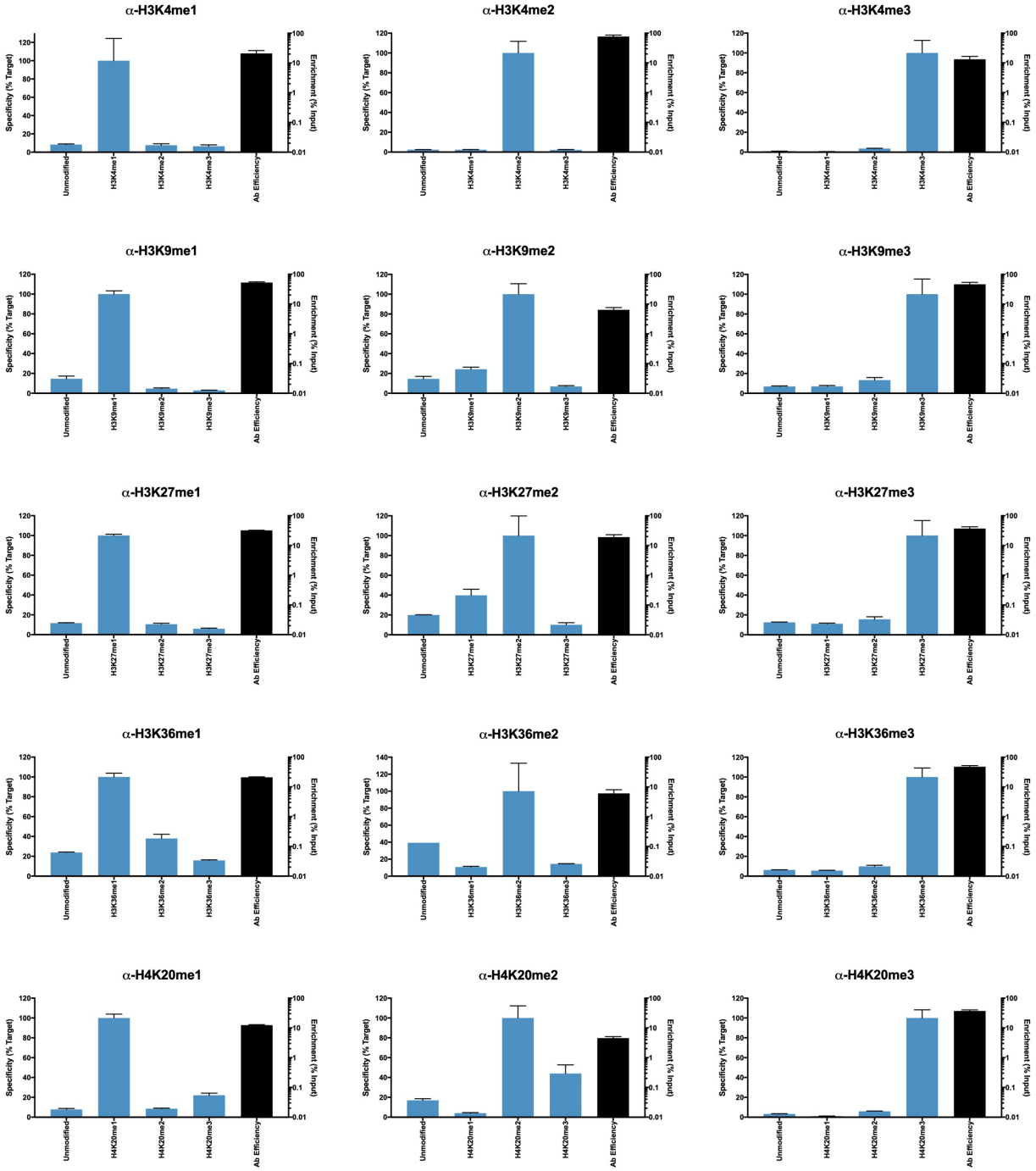
1. Grzybowski AT *et al* (2015) *Mol Cell* 58: 886-889
2. Shah RN *et al* (2018) *Mol Cell* 72:162-177
3. Janssen A *et al* (2019) *Genes Dev* 33:103-115
4. Lam KG *et al* (2019) *Nat Commun* 10: 3821



DNA Gel Data: Representative images for SNAP-ChIP K-MetStat nucleosomes (H3K4me0 = unmodified) resolved by native PAGE and stained with ethidium bromide to confirm intact nucleosome assembly with minimal free DNA. **Lane 1:** Free 147 bp DNA used in nuclesome assembly (100 ng). **Lane 2:** Intact nucleosomes (200 ng). Comparable experiments were performed for the entire K-MetStat Panel. Email techsupport@epicypher.com for more information.



Protein Gel Data: Representative Coomassie stained PAGE gel of SNAP-ChIP K-MetStat nucleosomes (2 µg each; H3K4me0 = unmodified) 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-MetStat Panel. For more information email techsupport@epicypher.com.



ChIP Data: Representative chromatin immunoprecipitation (ChIP) data using commercially available ChIP-grade antibodies targeting each PTM in the K-MetStat panel. The antibodies were assayed in a native ChIP experiment with 3 µg antibody added to 3 µg K-562 cell chromatin with the K-MetStat Panel spiked-in prior to micrococcal nuclease digestion. Quantitative real-time PCR (qPCR) was used to measure recovery of duplicate DNA barcodes corresponding to the indicated panel nucleosomes (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. Enrichment of off-target PTMs is due to antibody cross-reactivity. For more information, email techsupport@epicypher.com.