Histone H3K4me3 Antibody, SNAP-ChIP®

Catalog No. 13-0028
Lot No. 20279002-14
Pack Size 100 µg

EpiCypher®

Type	Monoclonal	**Host**	Rabbit
Target Size	15 kDa	**Reactivity**	H, M, WR
Format	Aff. Pur. IgG	**Appl.**	ChIP, ChIP-Seq, ELISA

Product Description:

This antibody meets EpiCypher's "SNAP-ChIP® Certified" criteria for specificity and efficient target enrichment in a ChIP experiment (<20% cross-reactivity across the panel, >5% recovery of target input). Histone H3 is one of the four proteins that are present in the nucleosome, the basic repeating subunit of chromatin, consisting of 147 base pairs of DNA wrapped around an octamer of core histone proteins (H2A, H2B, H3 and H4). This antibody reacts to H3K4me3 and no cross reactivity with other lysine methylations in the panel is detected.

Immunogen:

A synthetic peptide corresponding to histone H3 trimethylated at lysine 4.

Formulation:

Protein A affinity-purified antibody (1 mg/mL) in PBS, with 0.09% sodium azide, 1% BSA, and 50% glycerol.

Storage and Stability:

Stable for 1 year at -20°C from date of receipt.

Application Notes:

Recommended usage amounts:

ChIP 2 - 5 µg per 1x10^6 cells
ELISA 1 - 10 µg/mL

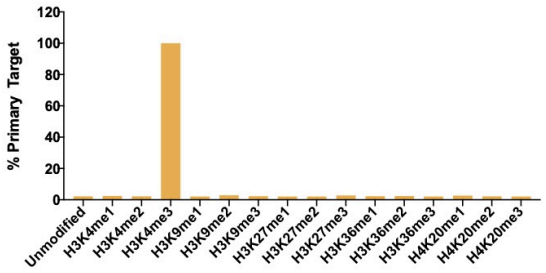

Figure 1: Luminex multiplexed specificity profiling. H3K4me3 antibody was assessed using a Luminex® based approach employing dCypher™ Nucleosome K-MetStat Panel (EpiCypher Catalog No. 16-9002). The panel comprises biotinylated designer nucleosomes (x-axis) individually coupled to color coded Luminex MagPlex® beads. Antibody binding to the panel of 16 nucleosomes was tested in multiplex at a 1:4000 dilution, and detected with second layer anti-IgG*PE. Data was generated using a Luminex FlexMAP3D®. Data is normalized to target (H3K4me3; set to 100).

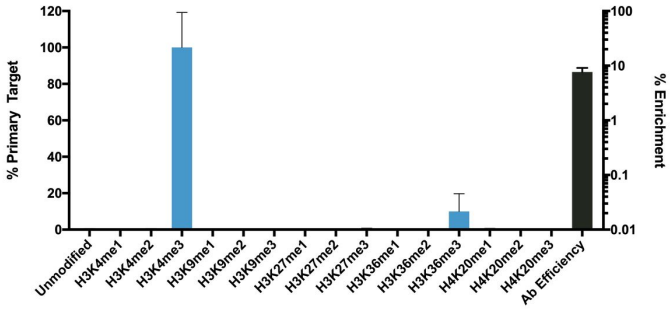

Figure 2: ChIP-qPCR. Histone H3K4me3 antibody (3 µg) was tested in a native ChIP experiment using chromatin from K-562 cells (3 µg) with the SNAP-ChIP K-MetStat Panel (EpiCypher Catalog No. 19-1001) spiked-in prior to micrococcal nuclease digestion. Specificity (left Y-axis) was determined by qPCR for the DNA barcodes corresponding to modified nucleosomes in the SNAP-ChIP panel (x-axis). Black bar represents antibody efficiency (right y-axis; log scale) and indicates percentage of the target immunoprecipitated relative to input. Error bars represent mean ± SEM in replicate ChIP experiments.

References:

Grzybowski et al (2015) Mol Cell 58:886
Shah et al (2018) Mol Cell 72:162

Applications Key: ChIP-Chromatin IP; ChIP-seq: Chromatin IP sequencing E-ELISA; FACS-Flow cytometry; IF-Immunofluorescence; IHC-Immunohistochemistry; ICC-Immunocytochemistry; IP-Immunoprecipitation; WB-Western Blotting; L-Luminex
Reactivity Key: B-Bovine; Ce-*C. elegans*; Ch-Chicken; Dm- *Drosophila*; Eu-Eukaryote; H-Human; M-Mouse; Ma-Mammal; R-Rat; Sc-*S.cerevesiae*; Sp-*S. pombe*; WR-Wide Range (predicted); X-Xenopus; Z-Zebrafish

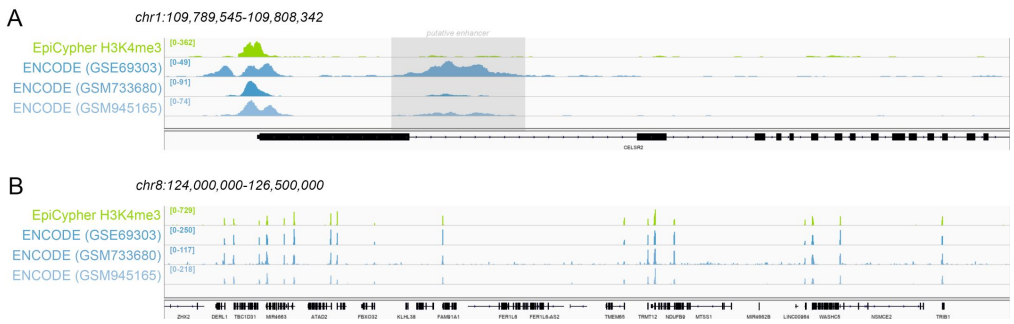

Figure 3: ChIP-seq. ChIP-seq was performed as described in Figure 2. Representative gene loci for EpiCypher H3K4me3 antibody ChIP-seq tracks (green tracks) are compared to three different ENCODE tracks (blue, GEO accession numbers noted in parentheses). EpiCypher H3K4me3 antibody shows specific enrichment at the *CELSR2* transcription start site (TSS), but no enrichment in the putative enhancer region (gray box) in contrast to several ENCODE tracks from antibodies known to cross-react with H3K4me2 (Shah et al., Mol Cell 2018) (**A**). A zoomed-out browser track shows H3K4me3 peaks at TSSs for multiple genes, with high signal:noise for the EpiCypher H3K4me3 antibody compared to three ENCODE tracks (note y-axis, **B**).

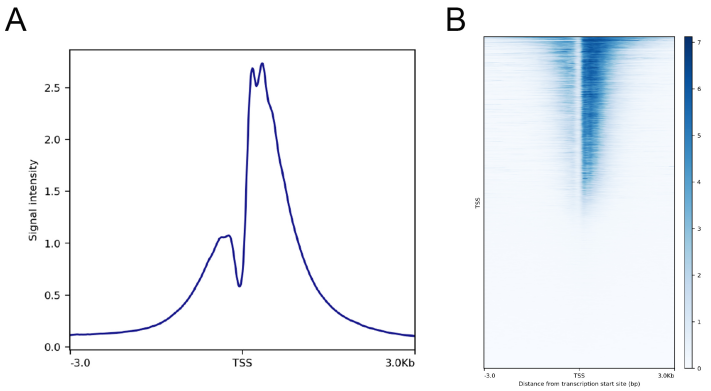

Figure 4: ChIP-seq. ChIP-seq was performed as described in Figure 2. Representative genome-wide analysis of H3K4me3 enrichment (signal intensity) flanking annotated TSSs is graphed as a cumulative histogram plot (**A**) as well as in a heatmap showing indivdual gene loci in each row colored by signal intensity and sorted by strongest to lowest enrichment (top to bottom; **B**). H3K4me3 displayed a characteristic enrichment pattern proximal to TSSs.

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