H3K9me3 Antibody - ChIP-seq Grade

Cat. No. C15410056

Type: Polyclonal ChIP grade, ChIP-seq grade	Specificity: Human, mouse, zebrafish, trout, Daphnia, Arabidopsis, Drosophila, silena latifolia: positive. Other species: not tested.
Size: 50 µg	Isotype: NA
Concentration: 1.85 µg/µl	Host: Rabbit
Lot No.: A2810P	Purity: Affinity purified polyclonal antibody.
Storage buffer: PBS containing 0.05% azide.	Storage conditions: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.	

Last Data Sheet Update: July 6, 2021

Description

Polyclonal antibody raised in rabbit against the region of histone **H3 containing the trimethylated lysine 9** (**H3K9me3**), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	0.5 - 1 µg per ChIP	Fig 1, 2
ELISA	1:1,000 - 1:10,000	Fig 3
Dot Blotting	1:2,000	Fig 4
Western Blotting	1:2,000	Fig 5

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5-5 µg per IP.

Target Description

Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble and are wrapped by 146 base pairs of DNA to form one octameric nucleosome. Histone tails undergo numerous post-translational modifications, which either directly or indirectly alter chromatin structure to facilitate transcriptional activation or repression or other nuclear processes. In addition to the genetic code, combinations of the different histone modifications reveal the so-called “histone code”. Histone methylation and demethylation is dynamically regulated by respectively histone methyl transferases and histone demethylases. Trimethylation of histone H3K9 is associated with satellite repeat regions and ZNF repeat genes.

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Validation data

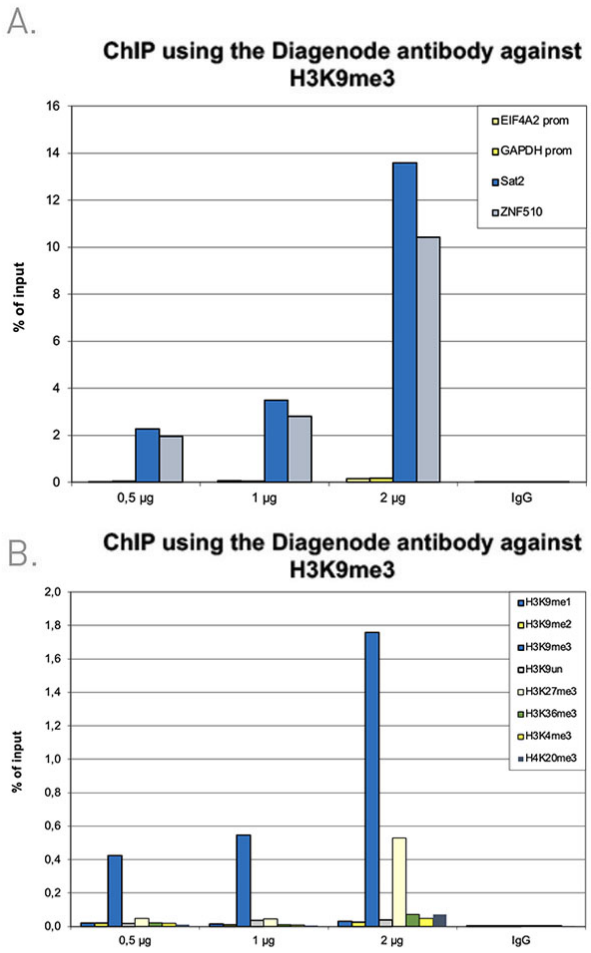

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K9me3

ChIP was performed with the Diagenode antibody against H3K9me3 (cat. No. C15410056) on sheared chromatin from 500,000 K562 cells using the "iDeal ChIP-seq" kit (cat. No. C01010051). The chromatin was spiked with a panel of in vitro assembled nucleosomes, each containing a specific lysine methylation (SNAP-ChIP K-MetStat Panel, Epicypher). A titration of the antibody consisting of 0.5, 1 and 2 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control.

Figure 1A. Quantitative PCR was performed with primers for the ZNF510 gene and the Sat2 satellite repeat, used as positive controls, and for the EIF4A2 and GAPDH promoters, used as negative controls. The graph shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

Figure 1B. Recovery of the nucleosomes carrying the H3K9me1, H3K9me2, H3K9me3, H3K4me3, H3K27me3, H3K36me3, H4K20me3 modifications and the unmodified H3K9 as determined by qPCR. The figure clearly shows the antibody is very specific in ChIP for the H3K9me3 modification when using 0.5 or 1 µg. With 2 µg of antibody, some recovery of the H3K27me3 nucleosome is observed.

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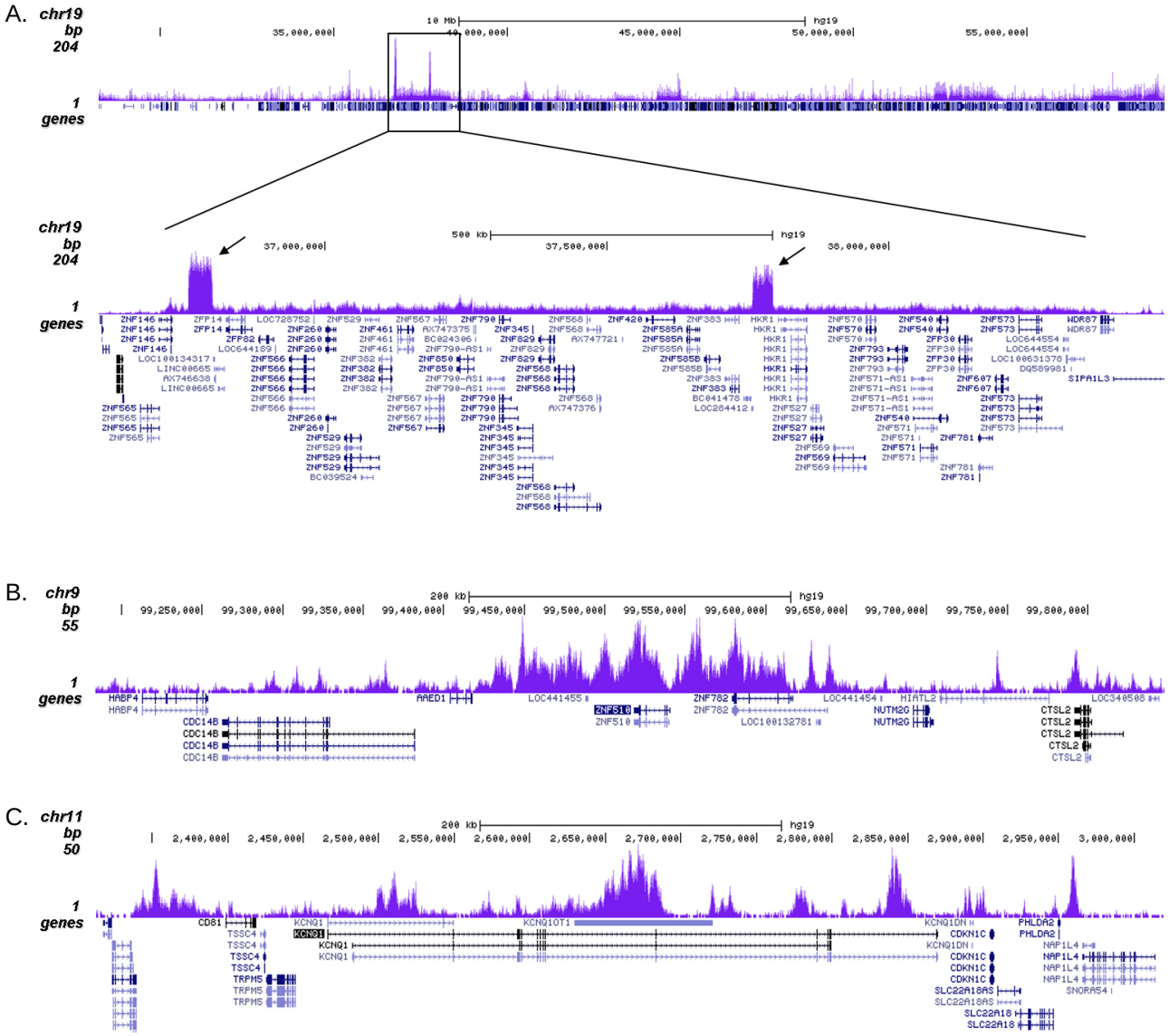

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K9me3

ChIP was performed with 1 µg of the Diagenode antibody against H3K9me3 (cat. No. C15410056) on sheared chromatin from 500,000 K562 cells using the "iDeal ChIP-seq" kit as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq4000. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2A shows the signal distribution along the long arm of chromosome 19 and a zoom-in to an enriched region containing several ZNF repeat genes. The arrows indicate two satellite repeat regions which exhibit a stronger signal. Figures 2B and 2C show the enrichment along the ZNF510 positive control target and at the KCNQ1 imprinted gene.

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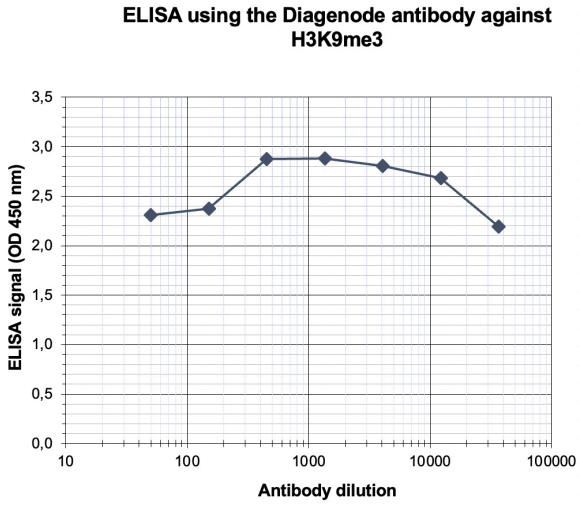

Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody directed against human H3K9me3 (cat. No. C15410056) in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:198,000.

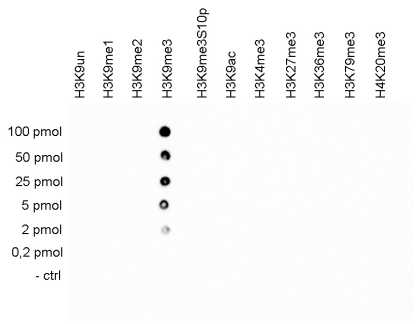

Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K9me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9me3 (cat. No. C15410056) with peptides containing other modifications of histone H3 and H4 and the unmodified sequence of histone H3. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:2,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

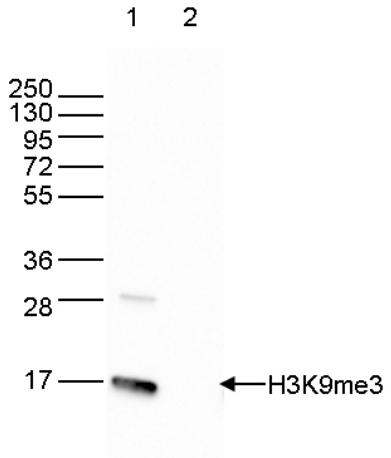

Figure 5. Western blot analysis using the Diagenode antibody directed against H3K9me3

Western blot was performed on 40 µg whole cell extracts from HeLa cells (lane 1) and on 1 µg of recombinant histone H3 (lane 2) using the Diagenode antibody against H3K9me3 (cat. No. C15410056). The antibody was diluted 1:2,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right, the marker (in kDa) is shown on the left.

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