H3K9me3 polyclonal antibody - Premium

Cat. No. C15410193

Type: Polyclonal	Specificity: Human, mouse, wide range expected
Size: 50 µg	Isotype: NA
Concentration: 0.9 µg/µl	Source: Rabbit
Lot No.: A0219P	Purity: Affinity purified polyclonal antibody.
Storage buffer: PBS containing 0.05% azide and 0.05% ProClin 300.	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: December 12, 2017

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 *	1 µg/ChIP	Fig 1, 2
ELISA	1:1,000	Fig 3
Dot Blotting/Peptide array	1:20,000/1:5,000	Fig 4
Western Blotting	1:1,000	Fig 5
Immunofluorescence	1:250	Fig 6

* 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 inactive genomic regions, satellite repeats and ZNF gene repeats.

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Validation Data

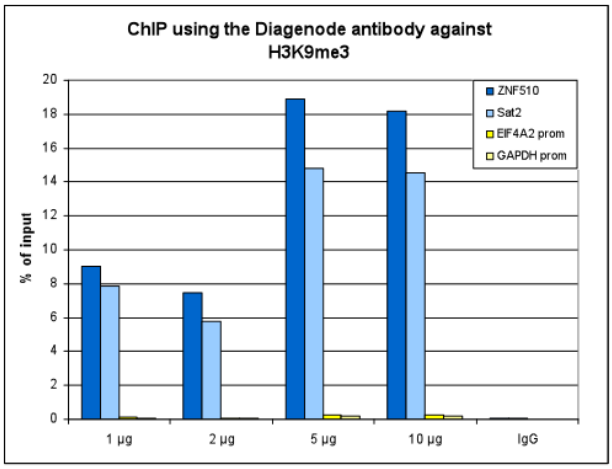
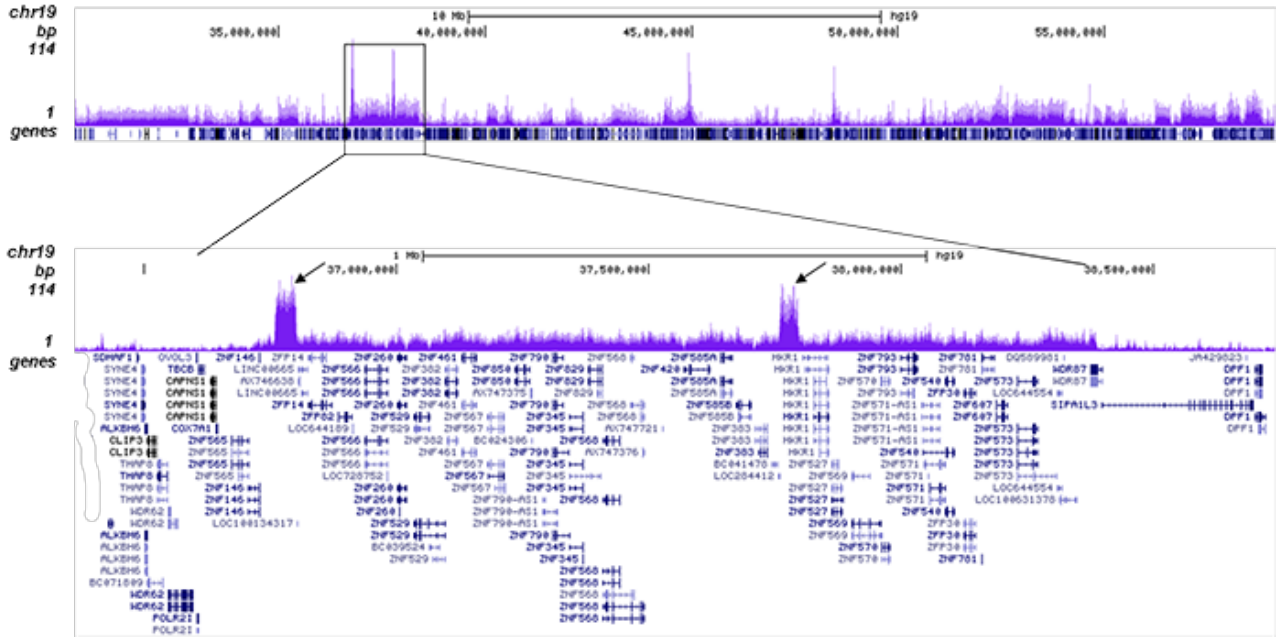


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K9me3

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K9me3 (Cat. No. C15410193) and optimized PCR primer pairs for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), using sheared chromatin from 1,000,000 cells. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoter of the active genes GAPDH and EIF4A2, used as negative controls, and for ZNF510 and the Sat2 satellite repeat, used as positive controls. The figure shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



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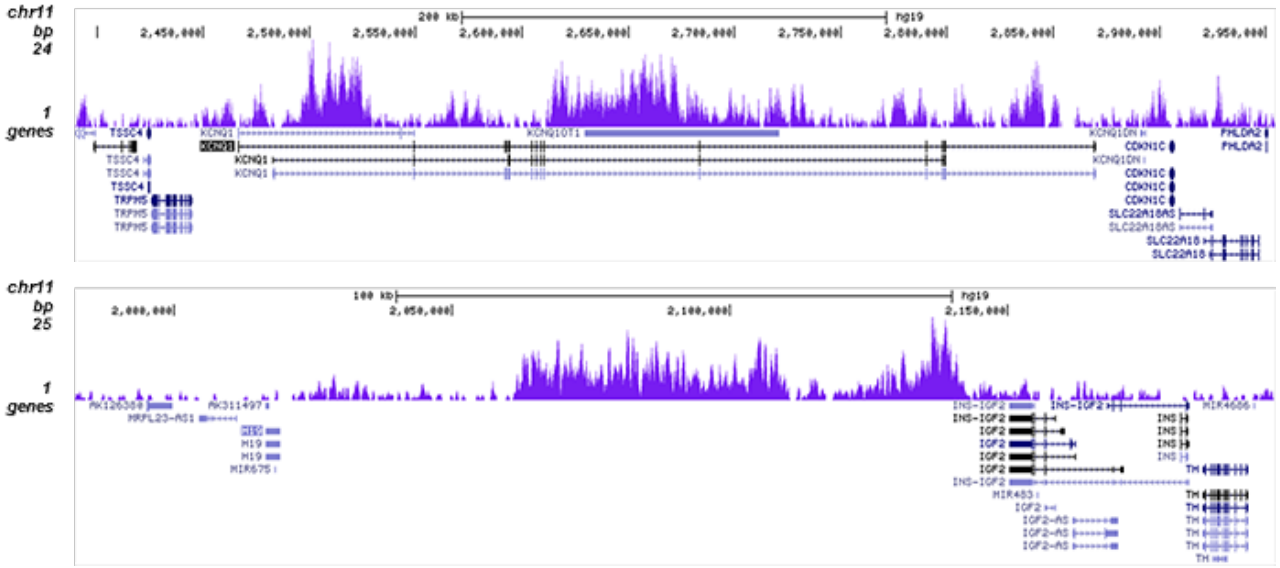

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K9me3

ChIP was performed with 1 µg of the Diagenode antibody against H3K9me3 (Cat. No. C15410193) on sheared chromatin from 1,000,000 HeLa cells using the "iDeal ChIP-seq" kit as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. 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 zoomin to an enriched region containing several ZNF repeat genes. The arrows indicate two satellite repeat regions which exhibit a stronger signal. Figure 2C and D show the enrichment at the KCNQ1 and H19 imprinted genes.

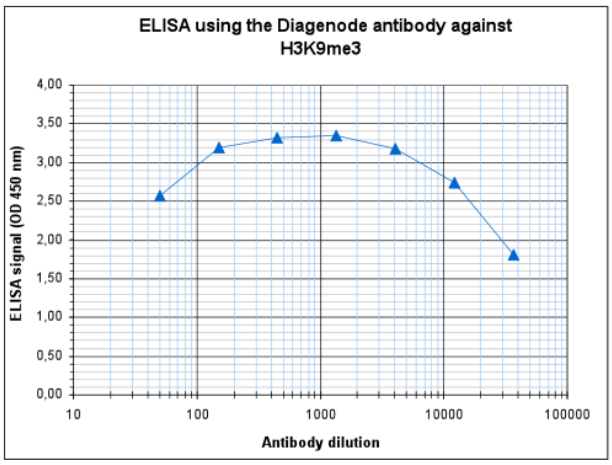

Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against H3K9me3 (Cat. No. C15410193). 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 1:42,700.

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A.

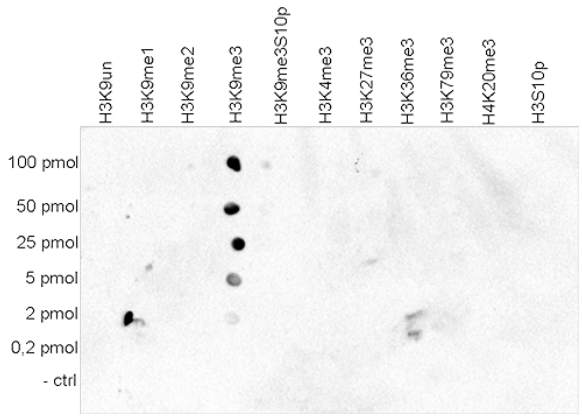
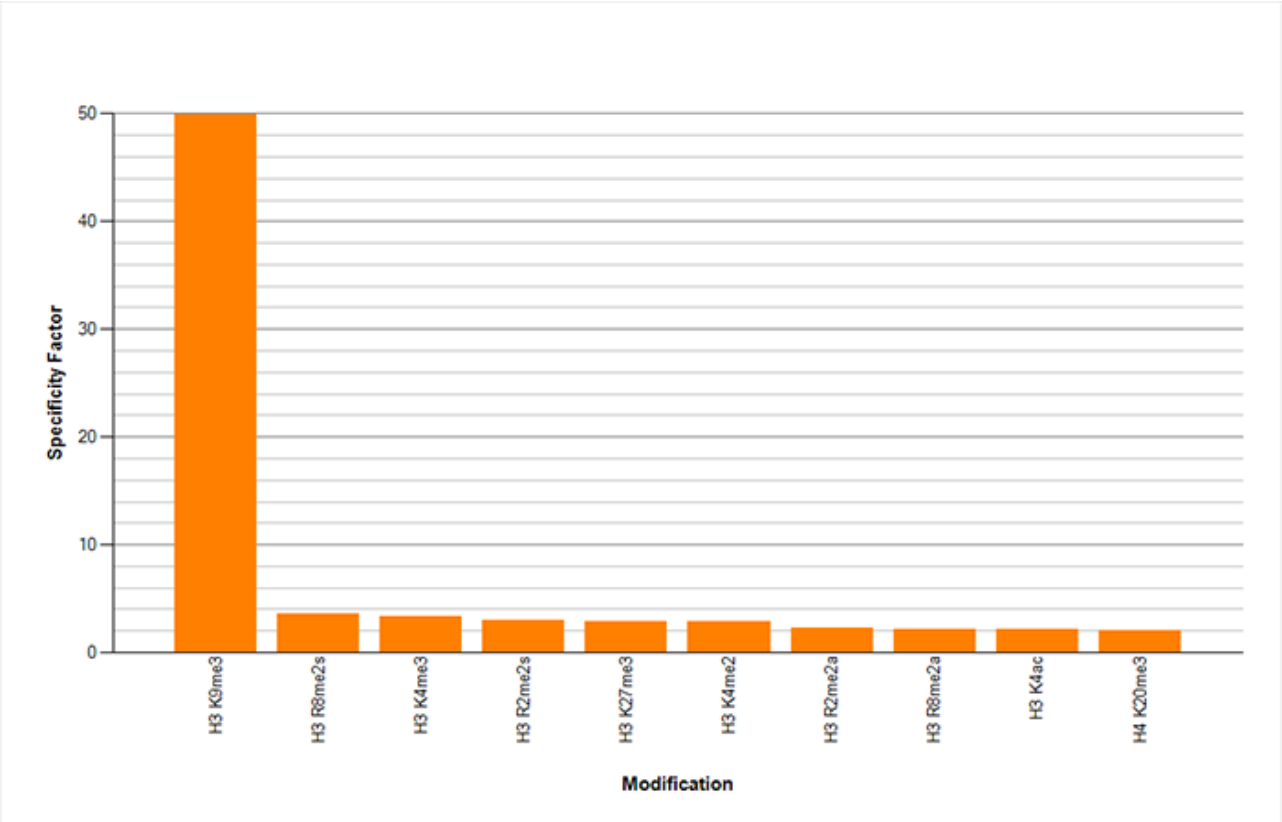


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K9me3

Figure 4A To test the cross reactivity of the Diagenode antibody against H3K9me3 (Cat. No. C15410193), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K9. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 4A shows a high specificity of the antibody for the modification of interest. Figure 4B The specificity of the antibody was further demonstrated by peptide array analyses on an array containing 384 peptides with different combinations of modifications from histone H3, H4, H2A and H2B. The antibody was used at a dilution of 1:5,000. Figure 4B shows the specificity factor, calculated as the ratio of the average intensity of all spots containing the mark, divided by the average intensity of all spots not containing the mark.

B.



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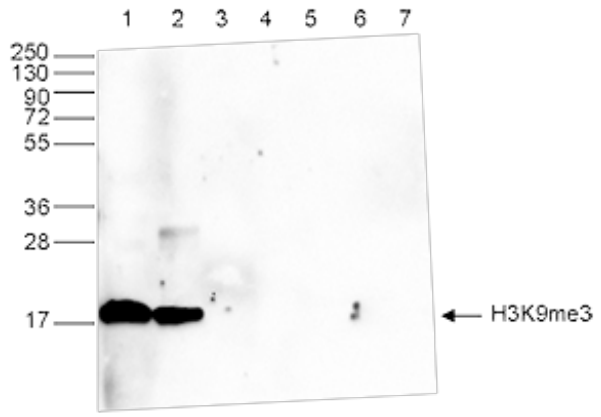

Figure 5. Western blot analysis using the Diagenode antibody directed against H3K9me3

Western blot was performed on whole cell (50 µg, lane 1) and histone extracts (15 µg, lane 2) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3.1, H3.2 and H4 (lane 3, 4, 5, 6 and 7, respectively) using the Diagenode antibody against H3K9me3 (Cat. No. C15410193). The antibody was diluted 1:1,000 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.

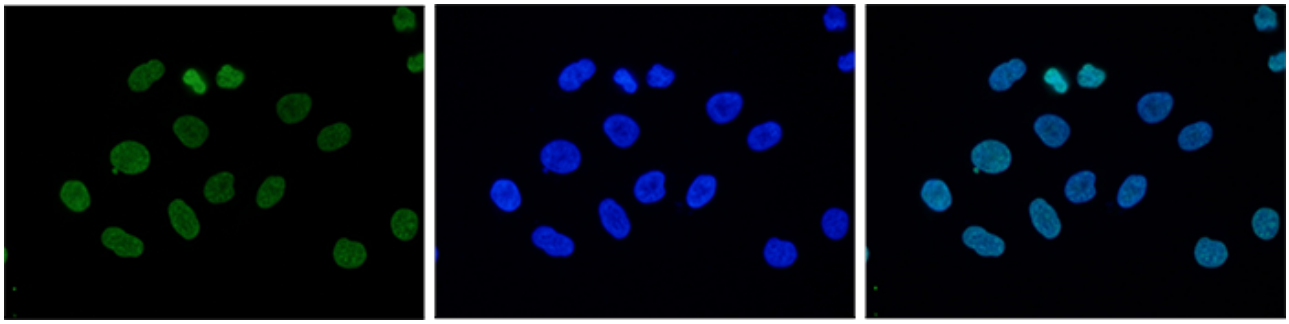

Figure 6. Immunofluorescence using the Diagenode antibody directed against H3K9me3

HeLa cells were stained with the Diagenode antibody against H3K9me3 (Cat. No. C15410193) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labeled with the H3K9me3 antibody (left) diluted 1:250 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.

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