H3K9me3 Antibody - ChIP-seq Grade

Cat. No. C15410193

Type: Polyclonal, **ChIP grade, ChIP-seq grade**	Specificity: Human, mouse, yeast, wide range expected
Size: 50 µg	Isotype: NA
Concentration: 1 µg/µl	Host: Rabbit
Lot No.: A2217P	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: January 14, 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/ChIP	Fig 1, 2
CUT&TAG	1 µg	Fig 3
ELISA	1:1,000 - 1:10,000	Fig 4
Dot Blotting	1:20,000	Fig 5
Western Blotting	1:1,000	Fig 6
Immunofluorescence	1:500	Fig 7

* 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.

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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

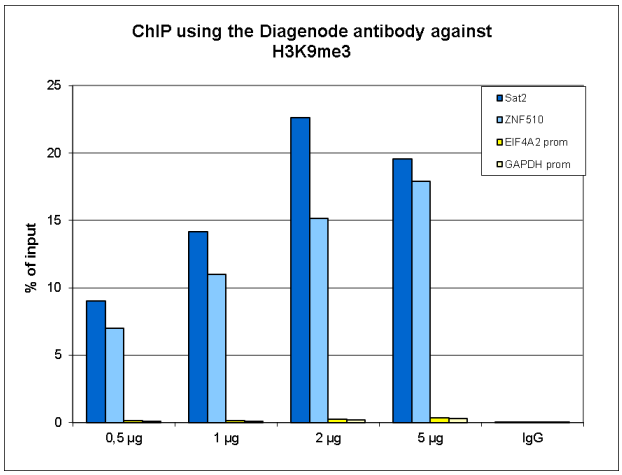
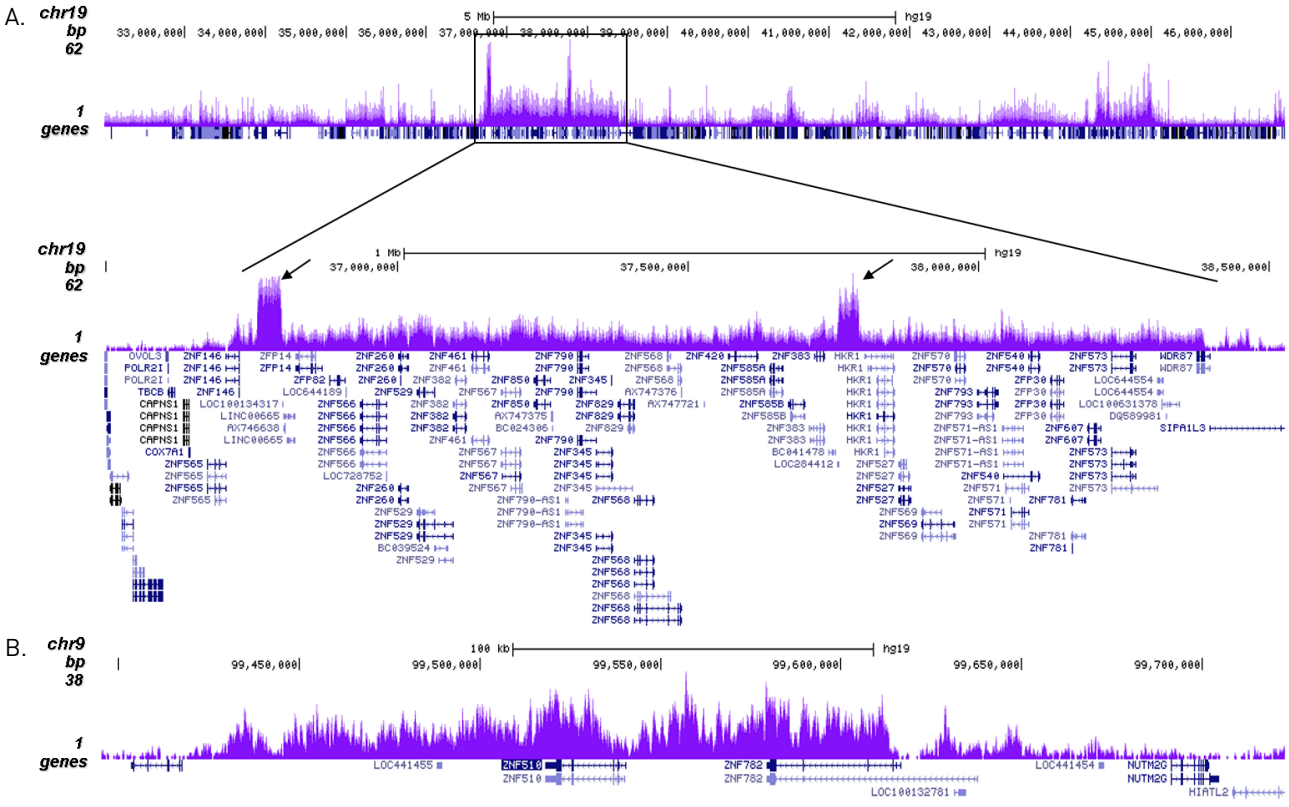


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K9me3

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K9me3 (cat. No. C15410193) and optimized PCR primer sets for qPCR. ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using the "iDeal ChIP-seq" kit (cat. No. C01010051). A titration of the antibody consisting of 0.5, 1, 2, and 5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the heterochromatin marker Sat2 and for the ZNF510 gene, used as positive controls, and for the promoters of the active EIF4A2 and GAPDH genes, used as negative controls. Figure 1 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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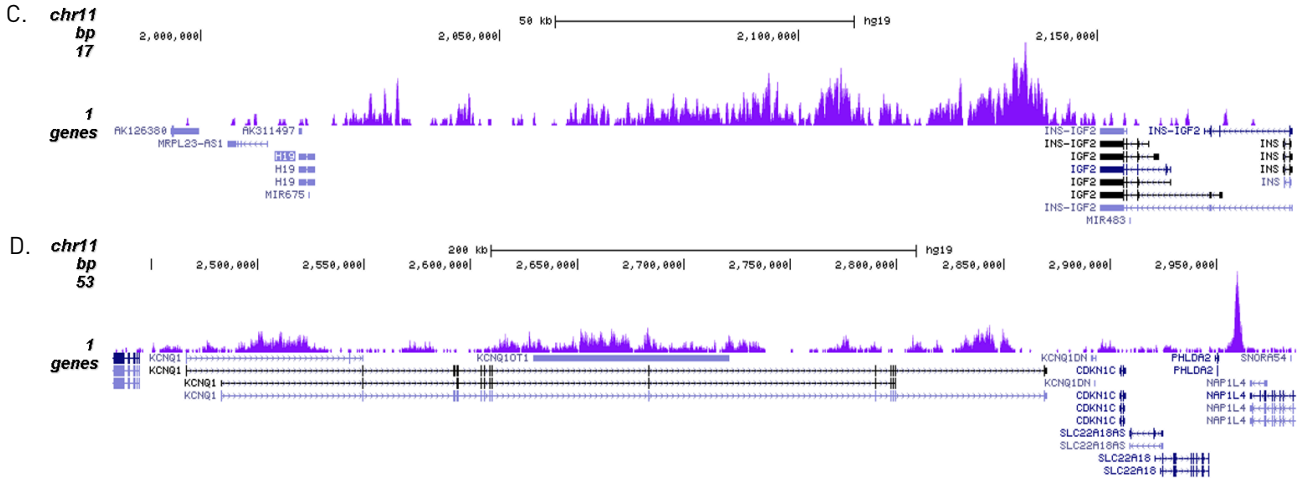

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K9me3

ChIP was performed with 0.5 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. Figures 2B, 2C and 2D show the enrichment along the ZNF510 positive control target and at the H19 and KCNQ1 imprinted genes.

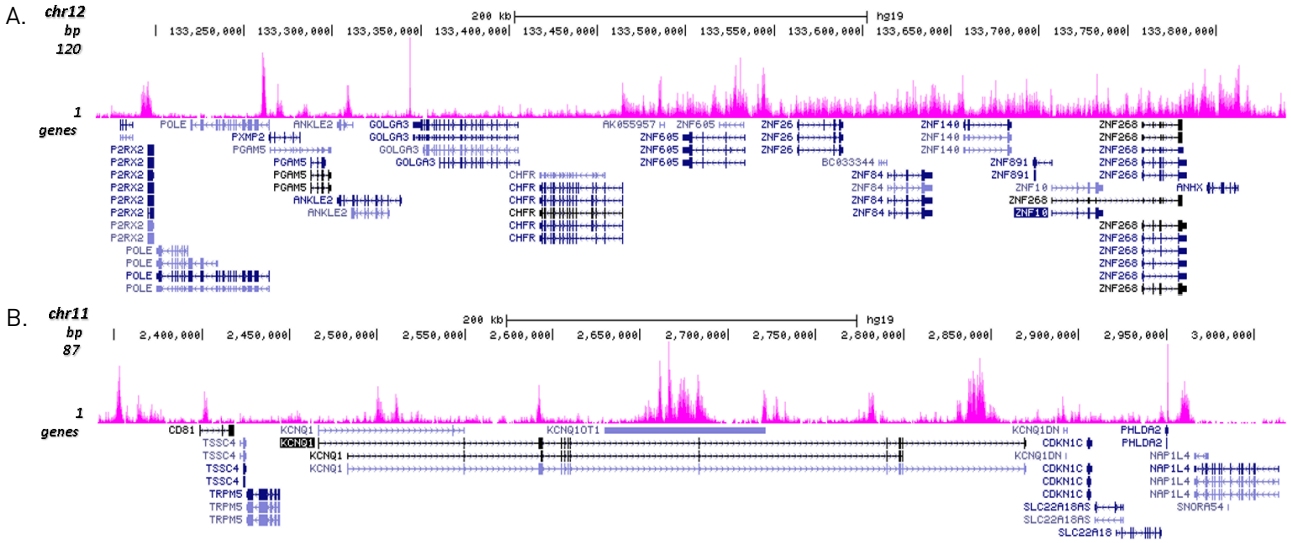

Figure 3. Cut&Tag results obtained with the Diagenode antibody directed against H3K9me3

CUT&TAG (Kaya-Okur, H.S., Nat Commun 10, 1930, 2019) was performed on 50,000 K562 cells using 1 µg of the Diagenode antibody against H3K9me3 (cat. No. C15410193) and the Diagenode pA-Tn5 transposase (C01070001). The libraries were subsequently analysed on an Illumina NextSeq 500 sequencer (2x75 paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg19) using the BWA algorithm. Figure 3 shows the peak distribution in a genomic region on chromosome 1 containing several ZNF repeat genes and in a genomic region surrounding the KCNQ1 imprinting control gene on chromosome 11 (figure 3A and B, respectively).

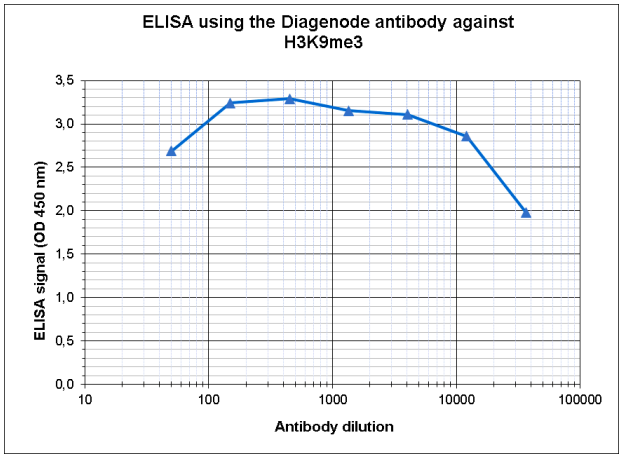

Figure 4. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody directed against human H3K9me3 (cat. No. C15410193) 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 4), the titer of the antibody was estimated to be 1:87,000.

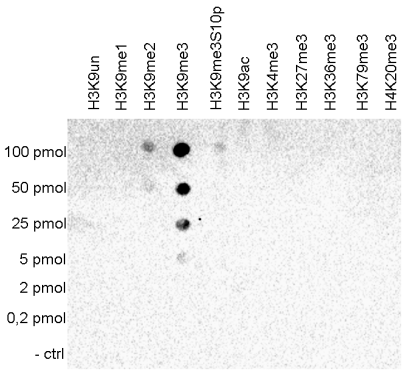

Figure 5. Cross reactivity tests using the Diagenode antibody directed against H3K9me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9me3 (cat. No. C15410193) with peptides containing other modifications and unmodified sequences of histone H3 and H4. 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 1:20,000. Figure 5 shows a high specificity of the antibody for the modification of interest.

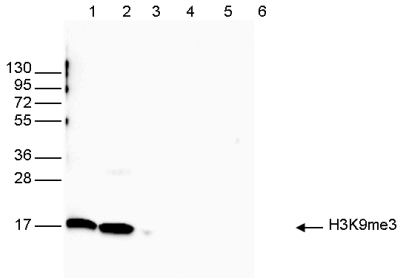

Figure 6. Western blot analysis using the Diagenode antibody directed against H3K9me3

Western blot was performed on whole cell (25 µg, lane 1) and histone extracts (15 µg, lane 2) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, 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.

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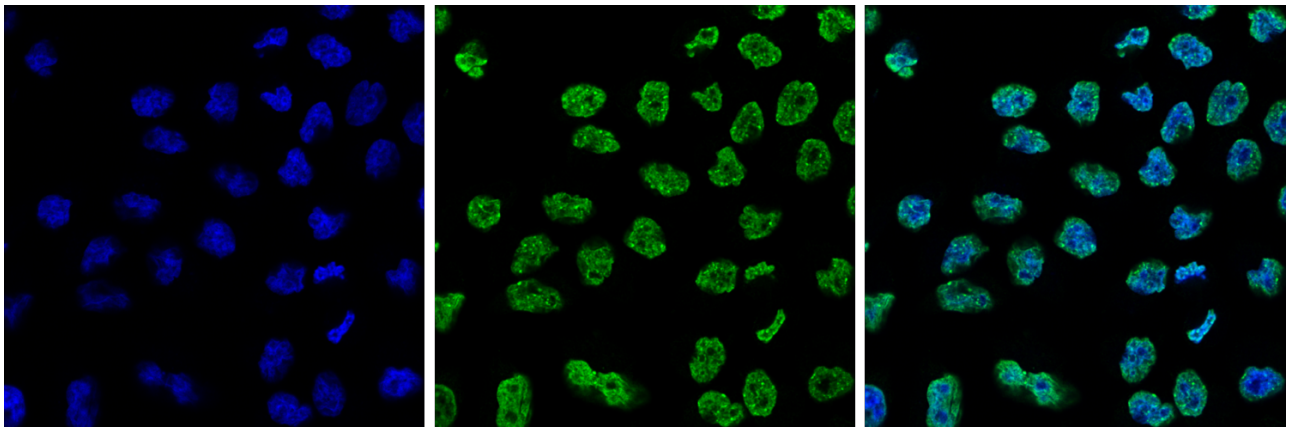

Figure 7. Immunofluorescence using the Diagenode antibody directed against H3K9me3

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 labelled with the H3K9me3 antibody (middle) diluted 1:500 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The left panel shows staining of the nuclei with DAPI. A merge of both stainings is shown on the right.

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