H3K27me3 Antibody - ChIP-seq Grade

Cat. No. C15410195

Type: Polyclonal	Specificity: Human, mouse, Drosophila, C. elegans, Daphnia, Arabidopsis, maize, tomato, poplar, silena latifolia, C. merolae, wide range expected.
Size: 50 µg	Isotype: NA
Concentration: 1.1 µg/µl	Host: Rabbit
Lot No.: A0824D	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 27** (**H3K27me3**), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	1 - 2 µg/IP per ChIP	Fig 1, 2
CUT&TAG	1 µg	Fig 3
ELISA	1:100 - 1:500	Fig 4
Dot Blotting/Peptide array	1:5,000	Fig 5
Western Blotting	1:1,000	Fig 6
Immunofluorescence	1:200	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.

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 alter chromatin structure to facilitate transcriptional activation, 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 regulated by histone methyl transferases and histone demethylases. Methylation of histone H3K27 is associated with inactive genomic regions.

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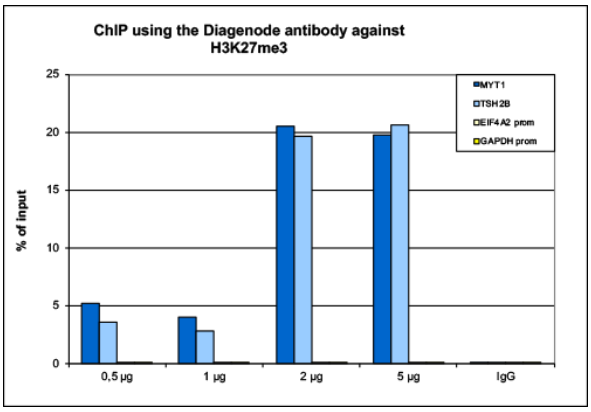
Validation Data

A.



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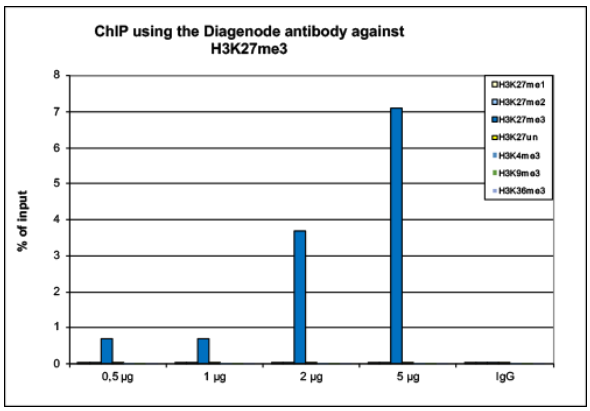


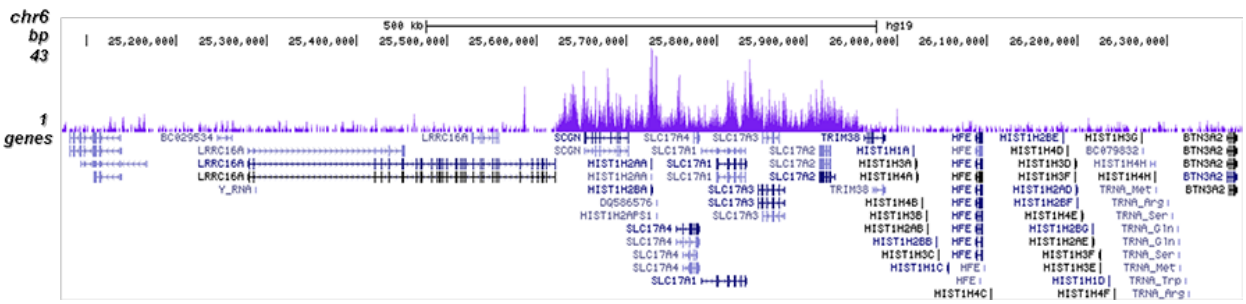
Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K27me3

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K27me3 (Cat. No. C15410195) and optimized PCR primer pairs for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), using sheared chromatin from 1 million cells. The chromatin was spiked with a panel of in vitro assembled nucleosomes, each containing a specific lysine methylation. A titration consisting of 0.5, 1, 2 and 5 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control.

Figure 1A. Quantitative PCR was performed with primers specific for the promoter of the active GAPDH and EIF4A2 genes, used as negative controls, and for the inactive TSH2B and MYT1 genes, used as positive 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 H3K27me1, H3K27me2, H3K27me3, H3K4me3, H3K9me3 and H3K36me3 modifications and the unmodified H3K27 as determined by qPCR. The figure clearly shows the antibody is very specific in ChIP for the H3K27me3 modification.

A.



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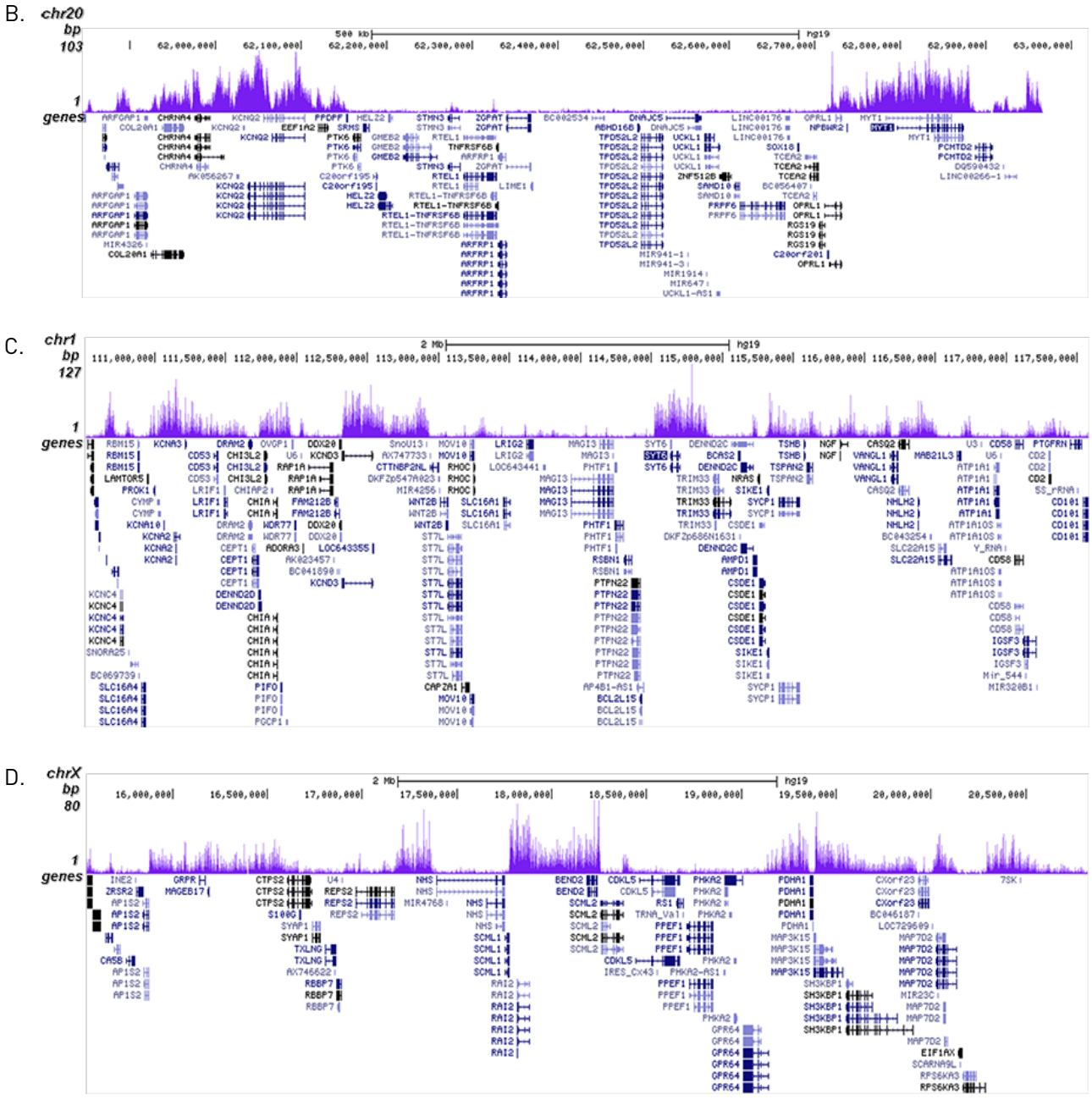

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K27me3

ChIP was performed on sheared chromatin from 1 million HeLa cells using 1 µg of the Diagenode antibody against H3K27me3 (Cat. No. C15410195) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. 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 2 shows the enrichment in genomic regions of chromosome 6 and 20, surrounding the TSH2B and MYT1 positive control genes (fig 2A and 2B, respectively), and in two genomic regions of chromosome 1 and X (figure 2C and D).

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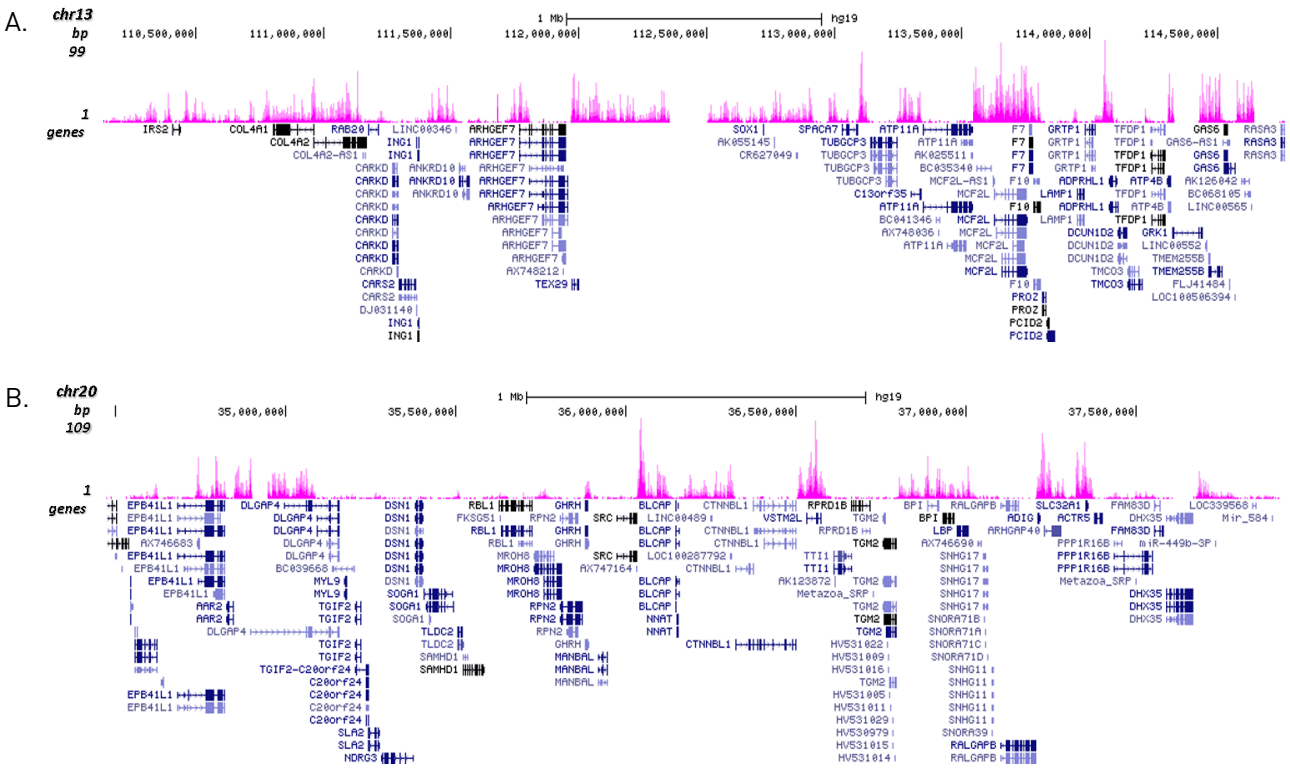

Figure 3. Cut&Tag results obtained with the Diagenode antibody directed against H3K27me3

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 H3K27me3 (cat. No. C15410195) 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 2 genomic regions on chromosome and 13 and 20 (figure 3A and B, respectively).

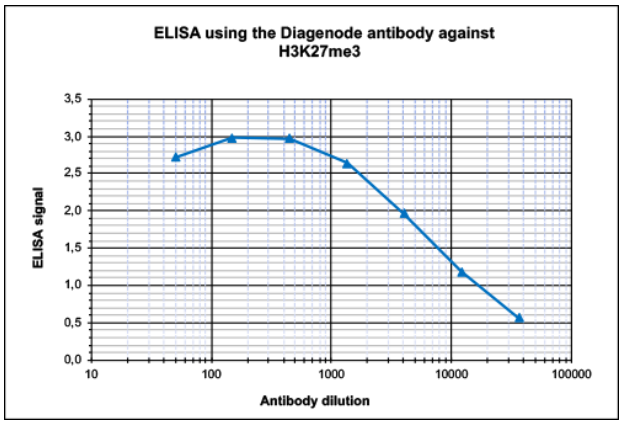

Figure 4. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K27me3 (Cat. No. C15410195). 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 1:3,000.

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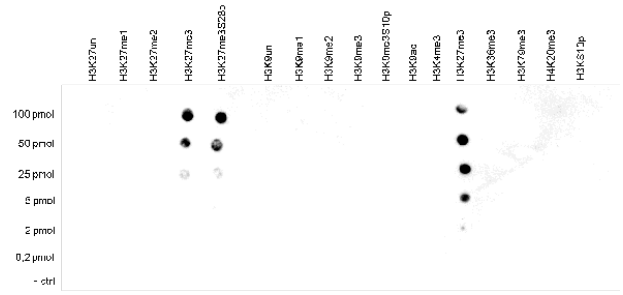

Figure 5. Cross reactivity tests using the Diagenode antibody directed against H3K27me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K27me3 (Cat. No. C15410195) with peptides containing other modifications of histone H3 and H4 and the unmodified H3K27 sequence. 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 1:5,000. Figure 5 shows a high specificity of the antibody for the modification of interest. Please note that the antibody also recognizes the modification if S28 is phosphorylated.

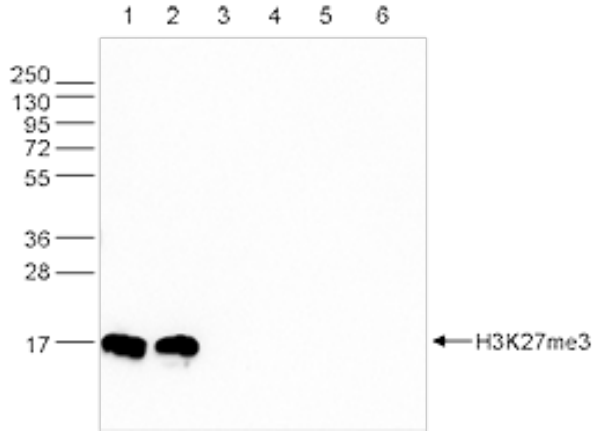

Figure 6. Western blot analysis using the Diagenode antibody directed against H3K27me3

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 H3K27me3 (cat. No. C15410195) diluted 1:500 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.

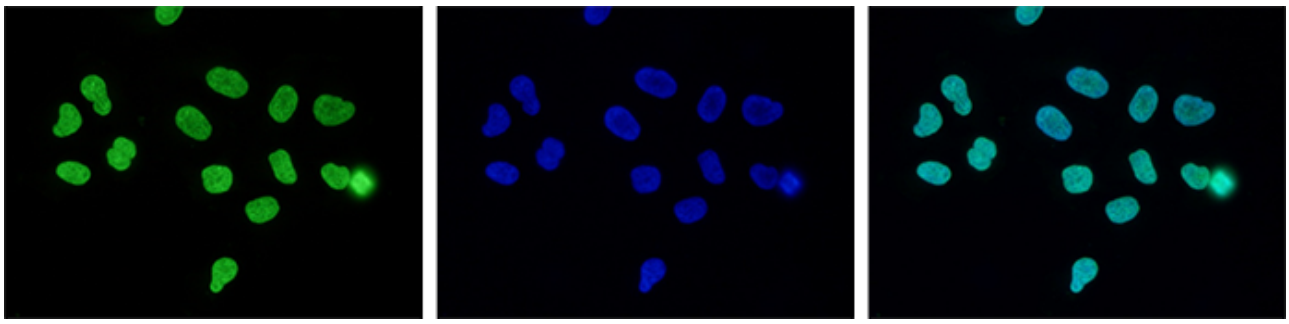

Figure 7. Immunofluorescence using the Diagenode antibody directed against H3K27me3

Human HeLa cells were stained with the Diagenode antibody against H3K27me3 (Cat. No. C15410195) 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 H3K27me3 antibody (left) diluted 1:200 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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