diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K27me3 antibody

Cat. No. C15410069

Type: Polyclonal, **ChIP/ChIP-seq grade**
Source: Rabbit
Lot: A1818P
Size: 50 µg
Concentration: 1.6 µg/µl

Specificity: Human, mouse, rat, pig, zebrafish, Drosophila, Schistosoma, Arabidopsis, cow
Purity: Affinity purified polyclonal antibody.
Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Storage buffer: PBS containing 0.05% azide and 0.05% ProClin 300.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Polyclonal antibody raised in rabbit against against histone H3, trimethylated at lysine 27 (H3K27me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	1 µg per ChIP	Fig 1, 2
ELISA	1:5,000	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:1,000	Fig 5
Immunofluorescence	1:500	Fig 6

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 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 H3K27 is associated with gene repression.

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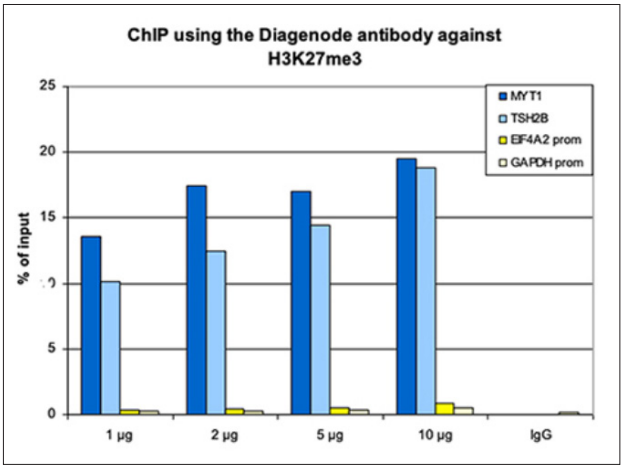
Results

A.



B.

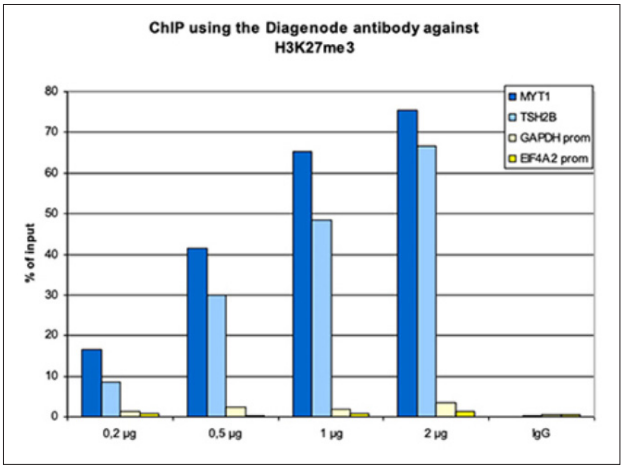
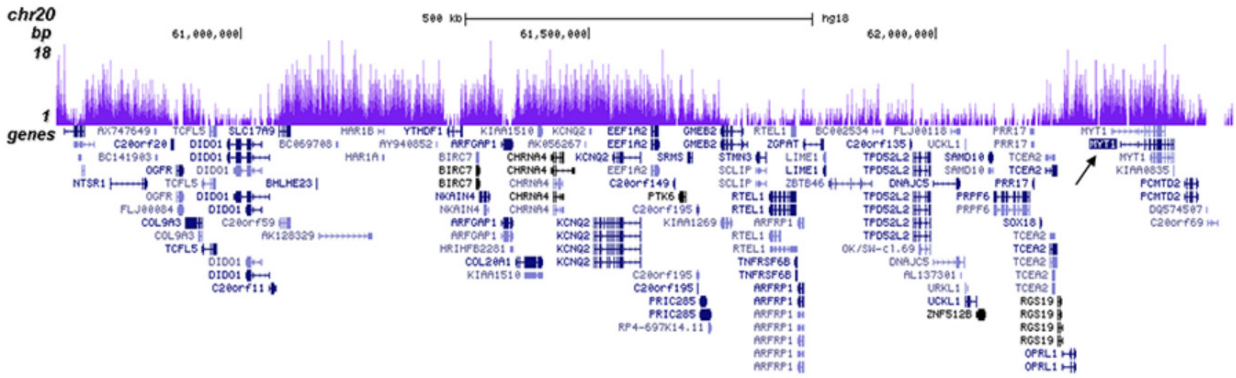


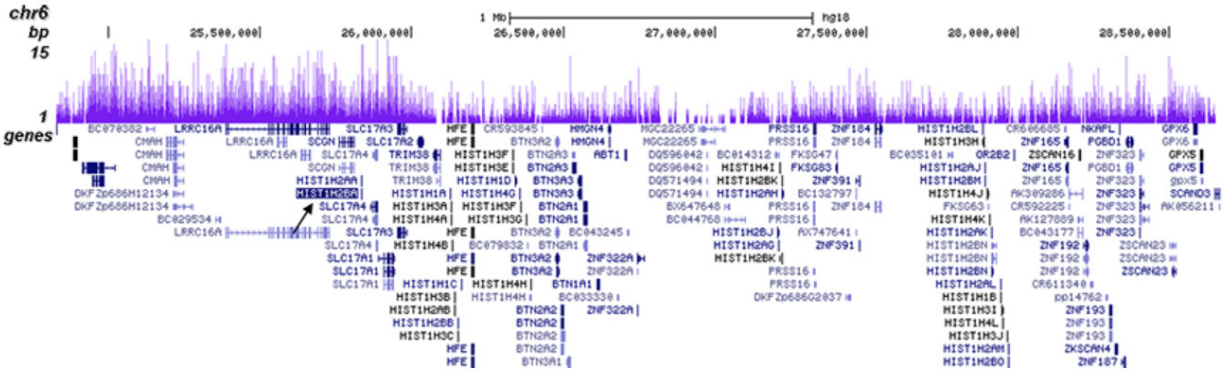
Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K27me3

ChIP assays were performed using human K562 cells, the Diagenode antibody against H3K27me3 (Cat. No. C15410069) 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 (figure A) or 100,000 cells (figure B). The indicated amounts of antibody were used per ChIP experiment. IgG (1 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 TSH2B and MYT1, 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).

A.



B.



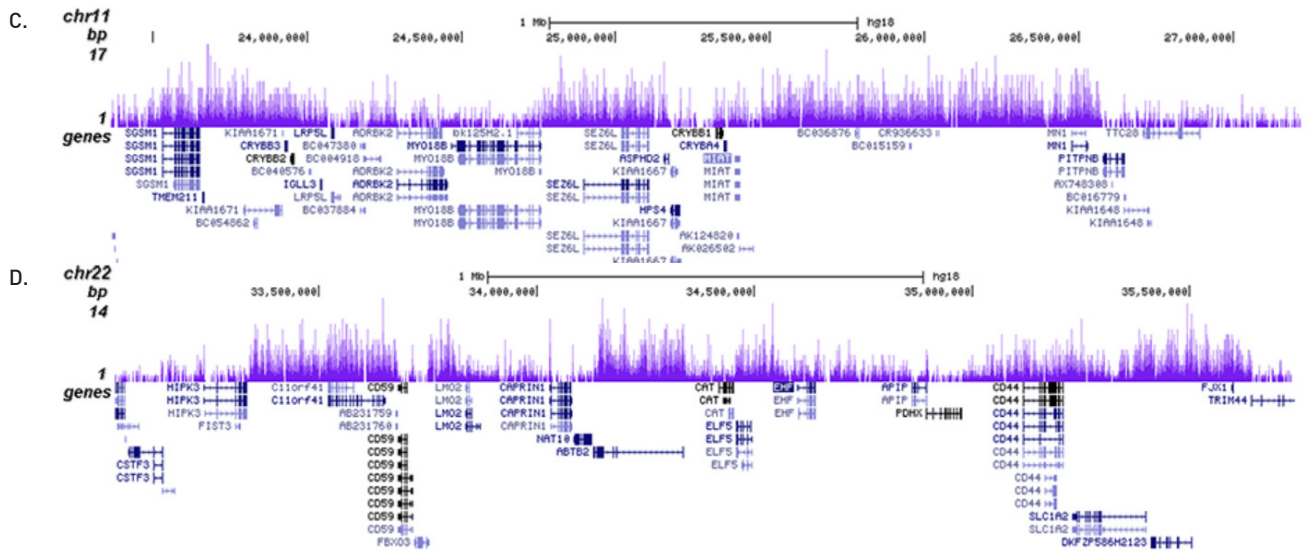


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K27me3

ChIP was performed on sheared chromatin from 100,000 K562 cells using 1 µg of the Diagenode antibody against H3K27me3 (Cat. No. C15410069) as described above. The IP'd DNA was subsequently analysed on an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure 2A and B show the signal distribution in two regions surrounding the MYT1 and TSH2B positive control genes, respectively. The position of the PCR amplicon, used for ChIP-qPCR is indicated with an arrow. Figure 2C and D show the signal distribution in two 3 Mb regions from chromosome 11 and 22.

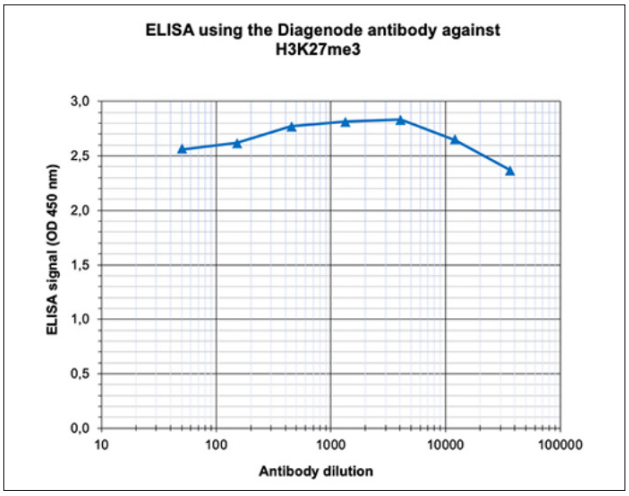


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against H3K27me3 (Cat. No. C15410069). 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 >1:1,000,000.

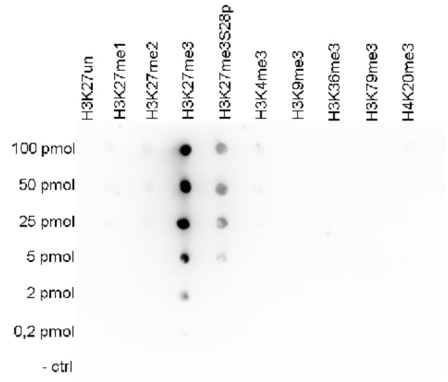


Figure 4. Cross reactivity test of the Diagenode antibody directed against H3K27me3

To test the cross reactivity of the Diagenode antibody against H3K27me3 (Cat. No. C15410069), a Dot Blot analysis was performed with peptides containing other modifications or unmodified sequences of histone H3 and H4. 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.

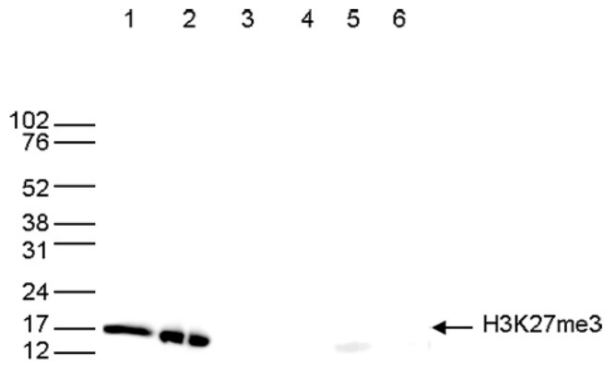


Figure 5. Western blot analysis using the Diagenode antibody directed against H3K27me3

Western blot was performed on whole cell (40 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. C15410069). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is shown on the right, the marker (in kDa) is shown on the left.

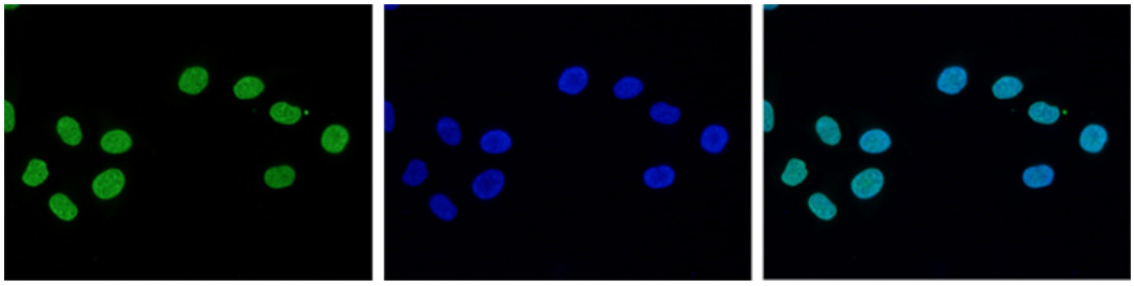


Figure 6. Immunofluorescence using the Diagenode antibody directed against H3K27me3

HeLa cells were stained with the Diagenode antibody against H3K27me3 (Cat. No. C15410069) 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 H3K27me3 antibody (left) diluted 1:500 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.