

H3K27me3S28p polyclonal antibody

Cat. No. C15410091

Type: Polyclonal ChIP-grade/ChIP-seq grade

Source: Rabbit

Lot #: A154-0011

Size: 50 µg/ 295 µl

Concentration: 0.17 µg/µl

Specificity: Human: positive

Other species: not tested

Purity: Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.

Storage: 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.

Description: Polyclonal antibody raised in rabbit against against histone H3, trimethylated at lysine 27 and phosphorylated at serine 28 (H3K27me3S28p), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP *	1-2 µg per ChIP	Fig 1, 2
ELISA	1:200	Fig 3
Dot blotting	1:2,000	Fig 4
Western blotting	1:1,000	Fig 5

* 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.

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Results

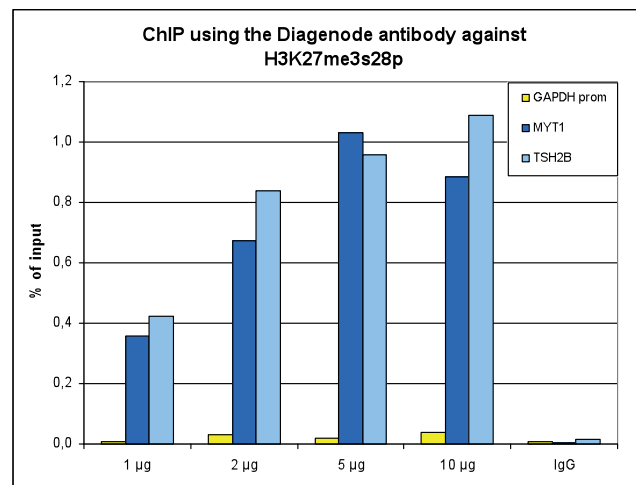


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K27me3S28p

ChIP assays were performed using human HeLa cells treated with colcemid, the Diagenode antibody against H3K27me3S28p (Cat. No. C15410091) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), using sheared chromatin from 1 million cells. A titration of the antibody consisting of 1, 2, 5, and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoter of the active gene GAPDH as a negative control, and for the coding regions of the inactive genes MYT1 and TSH2B as positive controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis). These results are in accordance with the observation that H3K27me3S28p is preferably present at inactive genes.

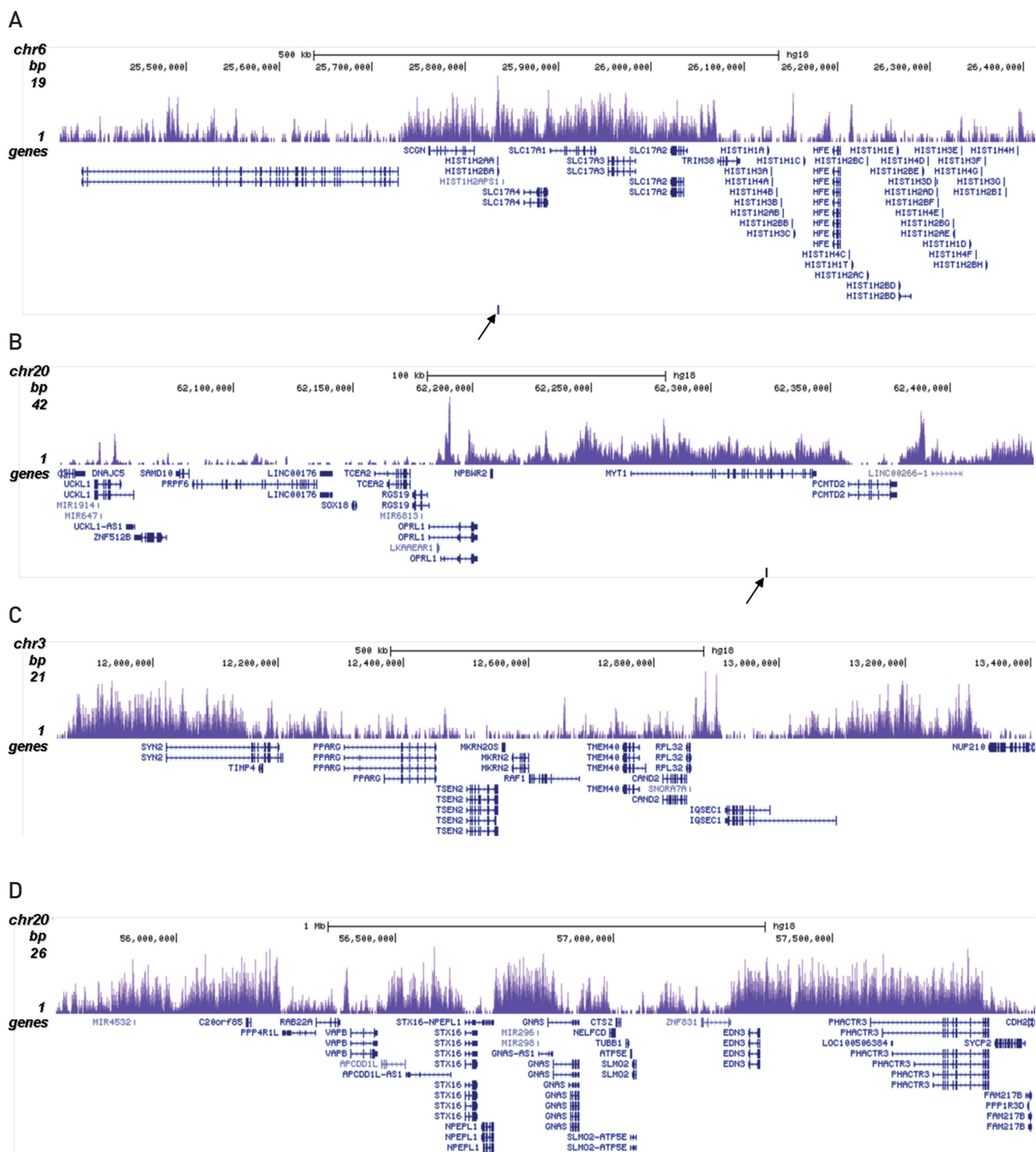


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K27me3S28p

ChIP was performed on sheared chromatin from 1.5 million colcemid treated HeLaS3 cells using 2 µg of the Diagenode antibody against H3K27me3S28p (Cat. No. C15410091) 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 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment in genomic regions surrounding the TSH2B and MYT1 positive control genes (fig 2A and B, indicated by an arrow), and of chromosome 3 and 20 (figure 2C and D).

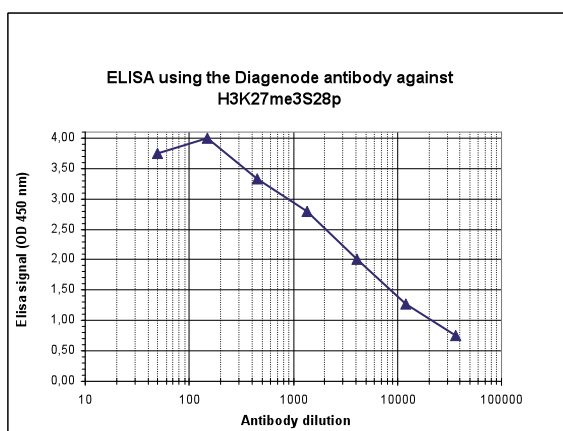


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K27me3S28p (Cat. No. C15410091). The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:4,300.

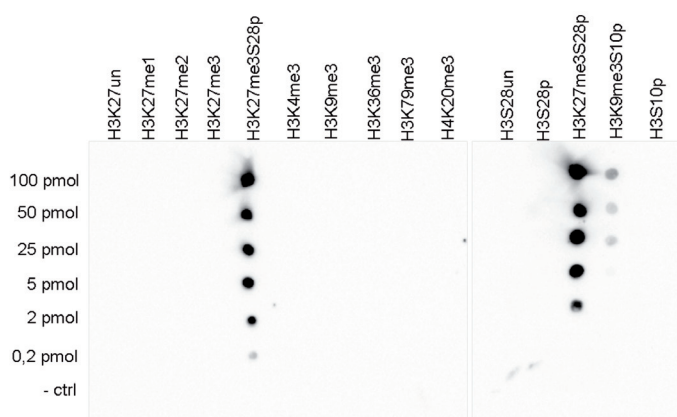


Figure 4. Cross reactivity test of the Diagenode antibody directed against H3K27me3S28p

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K27me3S28p (Cat. No. C15410091) with peptides containing other modifications of histone H3 and H4 and the unmodified H3K27S28 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 1:2,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

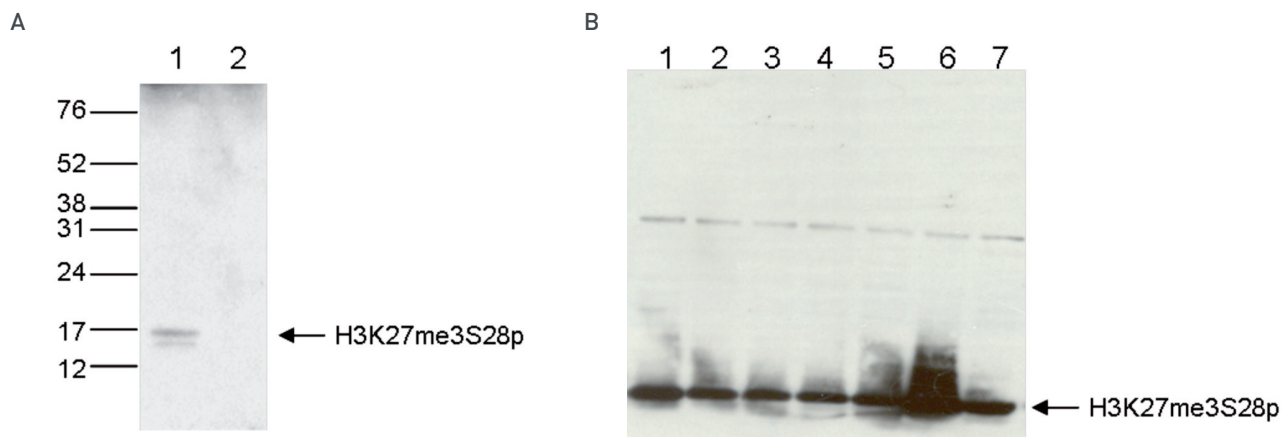


Figure 5. Western blot analysis using the Diagenode antibody directed against H3K27me3S28p

A. Western blot was performed on histone extracts (15 µg, lane 1) from HeLa cells, and on 1 µg of recombinant histone H3 (lane 2) using the Diagenode antibody against H3K27me3S28p (Cat. No. C15410091) diluted 1:100 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.

B. Cell cycle experiment using the Diagenode antibody against H3K27me3S28p. WB was performed on cells blocked in different stages of the cell cycle (1=asynchronous population, 2=G1/Sa, 3=G1/Sb, 4=S phase, 5=G2/M, 6=M, 7=G1). The picture shows a nice peak of H3K27me3S28p expression during mitosis.