diagenode
A Hologic Company

ChIP-seq grade

TECHNICAL DATASHEET

H3K27ac antibody

Cat. No. C15410174

Type: Polyclonal

Source: Rabbit

Lot: A7071-001P

Size: 50 µg

Concentration: 1.2 µg/µl

Specificity: Human, mouse, rat, pig: positive. Other species: not tested.

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 histone H3, acetylated at lysine 27 (H3K27ac), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	1 - 2 µg per ChIP	Fig 1, 2
ELISA	1:100	Fig 3
Dot blotting	1:25,000	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.

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Results

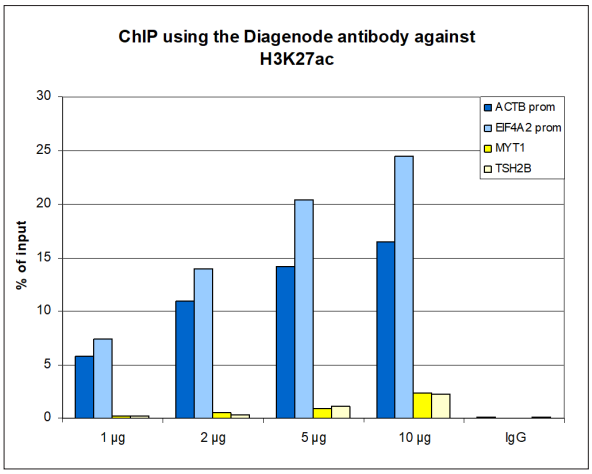
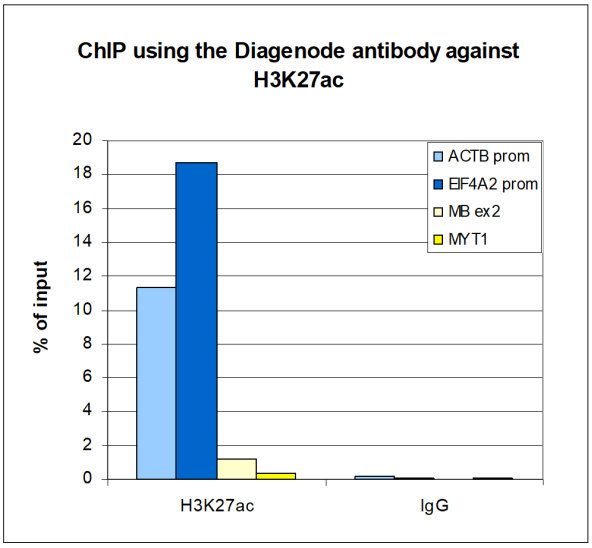


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K27ac

ChIP assays were performed using HeLa cells, the Diagenode antibody against H3K27ac (cat. No. C15410174) and optimized PCR primer sets for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit 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. Quantitative PCR was performed with primers for the promoter of the active ACTB and EIF4A2 genes, used as positive controls, and for the coding region of the inactive MYT1 and TSH2B 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). These results are in accordance with the observation that H3K27 acetylation is enriched at the promoters of active genes.

A.



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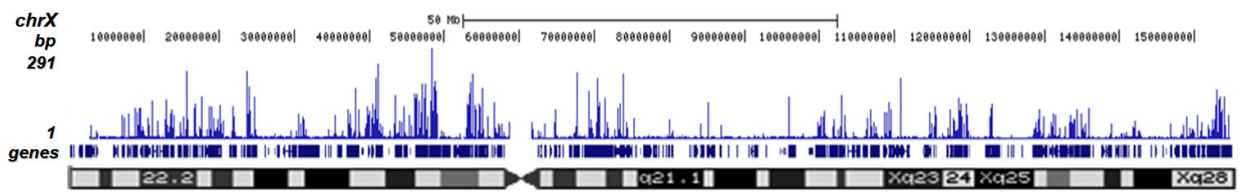

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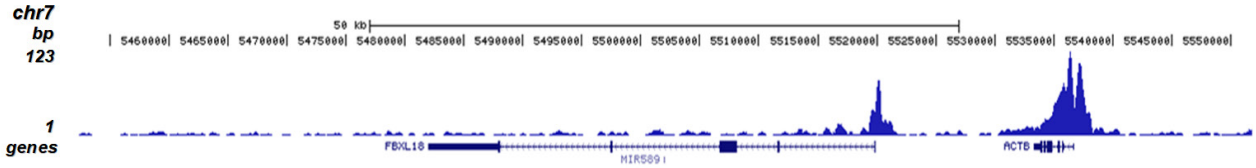

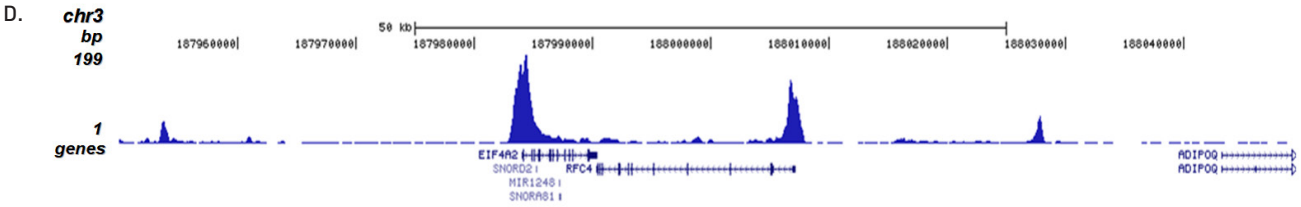

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K27ac

ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 2 µg of the Diagenode antibody against H3K27ac (cat. No. C15410174) as described above. Quantitative PCR was performed with primers for the promoter of the active ACTB and EIF4A2 genes, used as positive controls, and for the coding region of the inactive MYT1 and MB genes, used as negative controls (Figure 2A). 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 2B shows the peak distribution along the complete X-chromosome and in two regions surrounding the ACTB and EIF4A2 positive control genes, respectively (figure 2C and D).

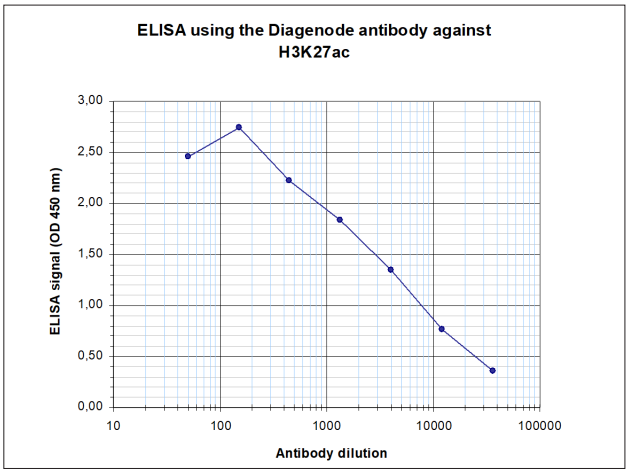

Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K27ac (cat. No. C15410174) 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 3), the titer of the antibody was estimated to be 1:4,000.

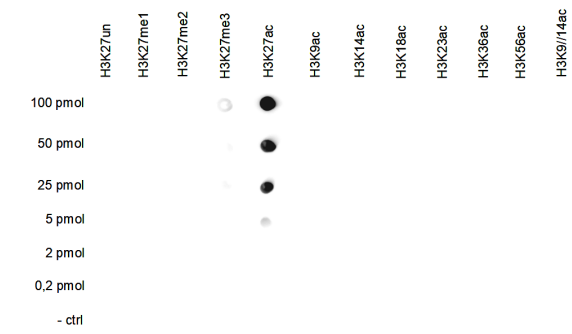

Figure 4. Cross reactivity test using the Diagenode antibody directed against H3K27ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K27ac (cat. No. C15410174) with peptides containing other histone modifications and the unmodified H3K27 sequence. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:25,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

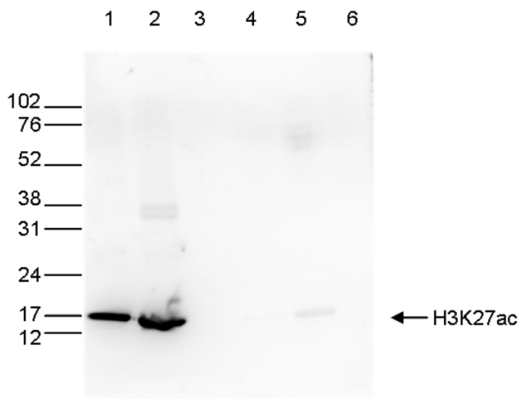

Figure 5. Western blot analysis using the Diagenode antibody directed against H3K27ac

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 H3K27ac (cat. No. C15410174). 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.

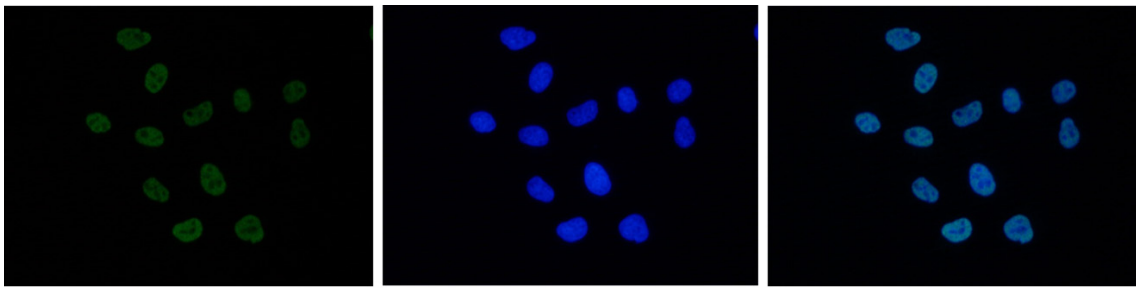

Figure 6. Immunofluorescence using the Diagenode antibody directed against H3K27ac

HeLa cells were stained with the Diagenode antibody against H3K27ac (cat. No. C15410174) 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 H3K27ac 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.