diagenode
A Hologic Company

ChIP-seq grade

TECHNICAL DATASHEET

H3K9ac antibody

Cat. No. C15410004

Type: Polyconal

Source: Rabbit

Lot: A1435-0012D

Size: 50 µg

Concentration: 1.35 µg/µl

Specificity: Human, mouse, pig, zebrafish, Poplar, Daphnia, P. Falciparum: positive.

Purity: Affinity purified.

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 9 (H3K9ac), using a KLHconjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	1-2 µg per ChIP	Fig 1, 2
ELISA	1:1,000	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.

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Results

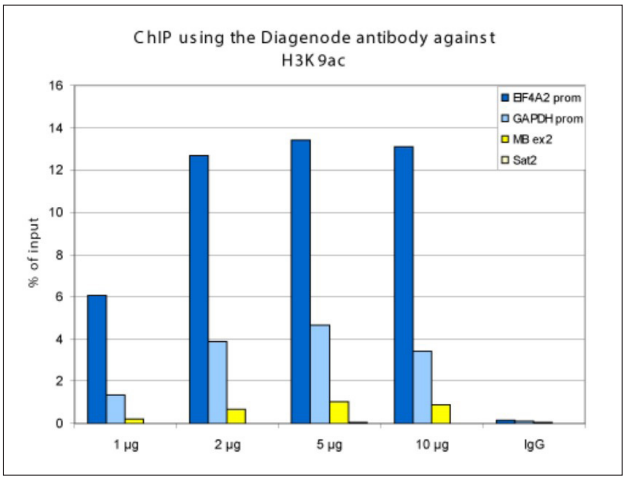

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K9ac

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K9ac (Cat. No. C15410004) 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. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers speci c for the promoter of the active genes GAPDH and EIF4A2, used as positive controls, and for exon 2 of the inactive myoglobin (MB) gene and the Sat2 satellite repeat, 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 acetylation of K9 at histone H3 is associated with the promoters of active genes.

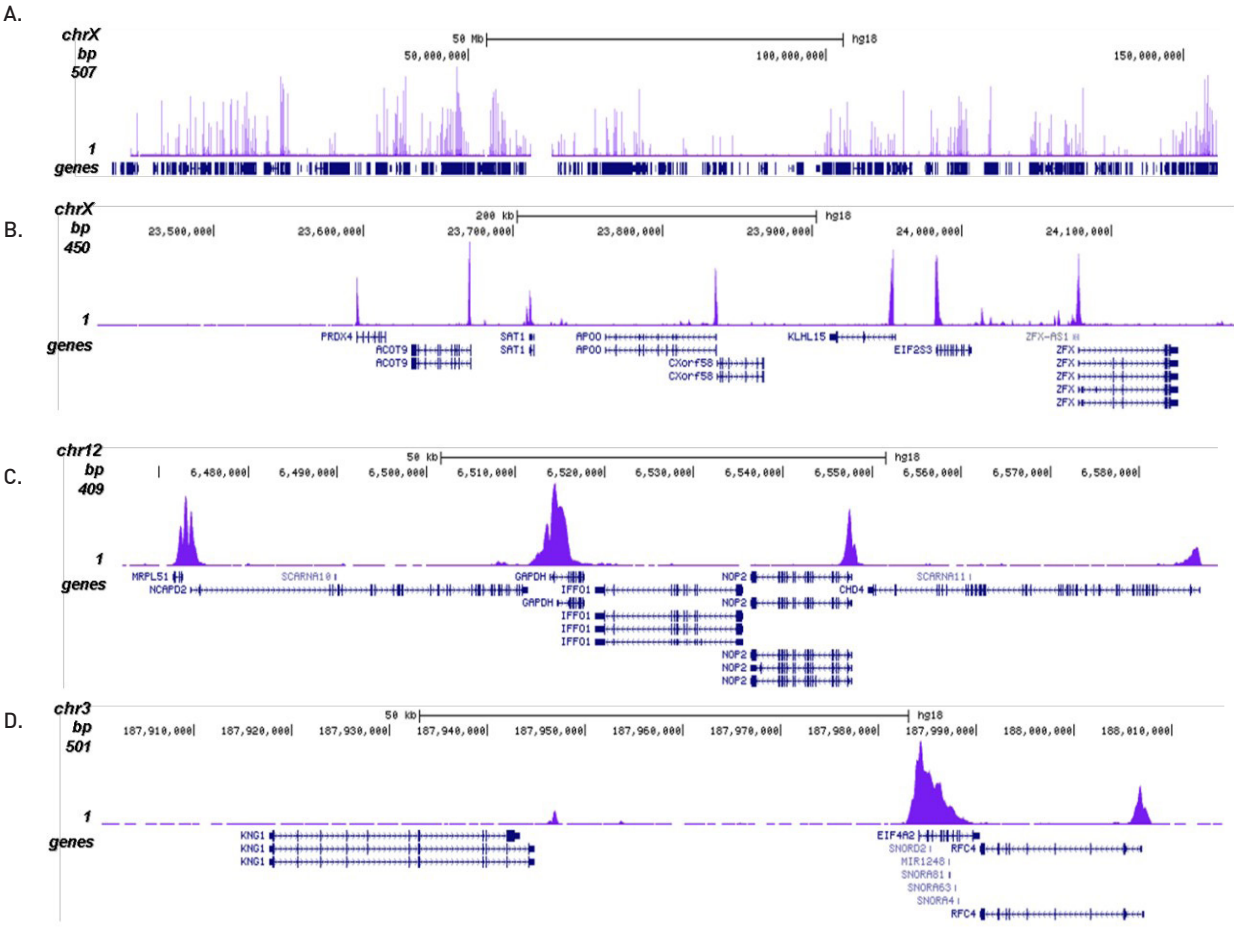

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K9ac

ChIP was performed with 1 µg of the Diagenode antibody against H3K9ac (Cat. No. C15410004) as described above and 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 2 shows the peak distribution along the complete sequence and an 800 kb region of the X-chromosome (gure 2A and B) and in 100 kb regions surrounding the GAPDH and EIF4A2 positive control genes (gure 2C and D). These results clearly show an enrichment of H3K9ac at the promoters of active genes.

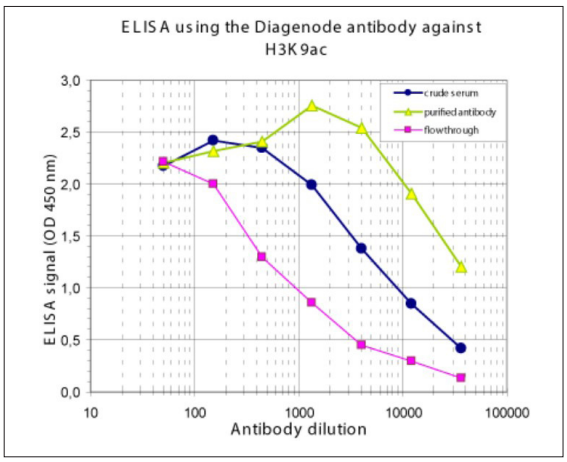

Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K9ac (Cat. No. C15410004) in antigen coated wells. The antigen used was a peptide containing the histone modi cation of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:31,700.

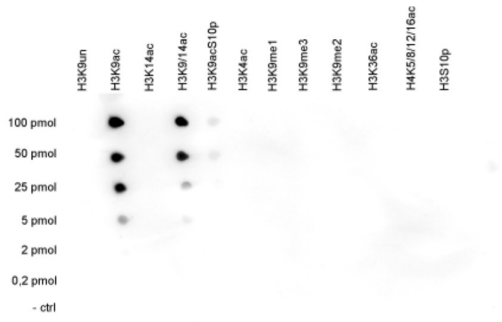

Figure 4. Cross reactivity test using the Diagenode antibody directed against H3K9ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9ac (Cat. No. C15410004) with peptides containing other histone modi cations and the unmodi ed H3K9 sequence. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 4 shows a high speci city of the antibody for the modi cation of interest. Please note that this antibody recognizes the H3K9 acetylation, both in the presence and the absence of the K14 acetylation.

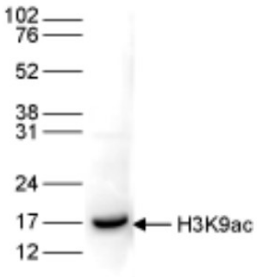

Figure 5. Western blot analysis using the Diagenode antibody directed against H3K9ac

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K9ac (Cat. No. C15410004) 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.

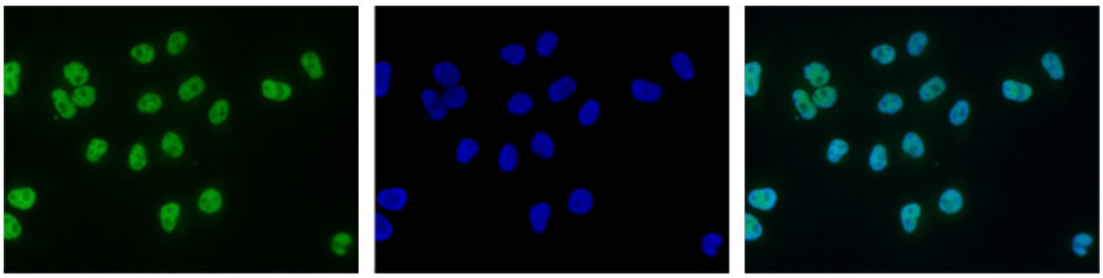

Figure 6. Immunofluorescence using the Diagenode antibody directed against H3K9ac

HeLa cells were stained with the Diagenode antibody against H3K9ac (Cat. No. C15410004) and with DAPI. Cells were xed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immuno uorescently labelled with the H3K9ac 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.