diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K4ac polyclonal antibody - Classic

Cat. No. C15410322

Type: Polyclonal	Specificity: Human
Size: 50 µg	Isotype: NA
Concentration: 1.1 µg/µl	Host: Rabbit
Lot No.: A2505P	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.	

Description

Polyclonal antibody raised in rabbit against histone H3 acetylated at lysine 4 (H3K4ac), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	2 µg/ChIP	Fig 1, 2
ELISA	1:1,000 - 1:10,000	Fig 3
Dot Blotting	1:5,000	Fig 4
Western Blotting	1:1,000	Fig 5
Immunofluorescence	1:200	Fig 6

* Please note that the optimal antibody amount per IP should be determined by the end-user.

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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Validation data

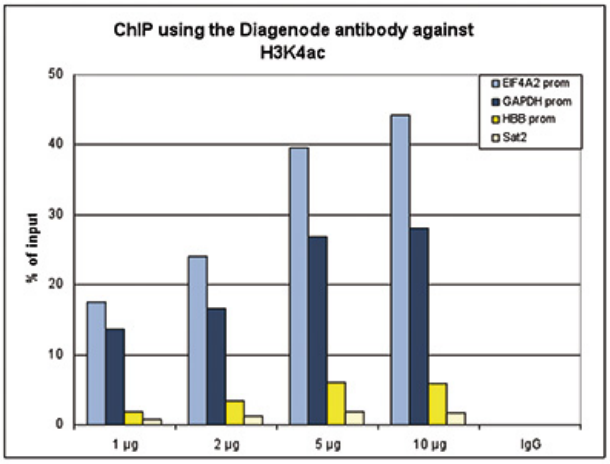
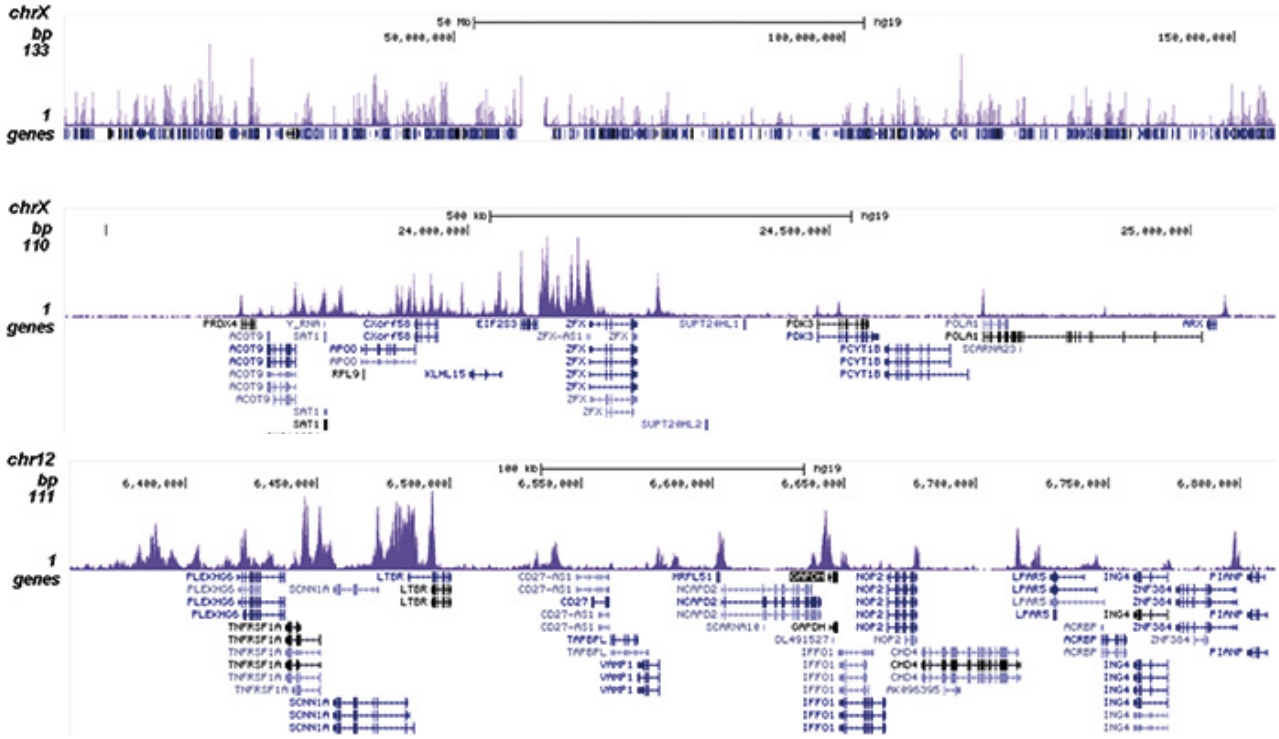


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4ac

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K4ac (Cat. No. C15410322) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), using sheared chromatin from 4 million cells. A titration of the antibody consisting of 1, 2, 5 and 10 µg per ChIP experiment was analysed. IgG (1 ?g/IP) was used as negative IP control. QPCR was performed with primers for the GAPDH and EIF4A2 promoters, used as positive controls, and for the HBB promoter 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).



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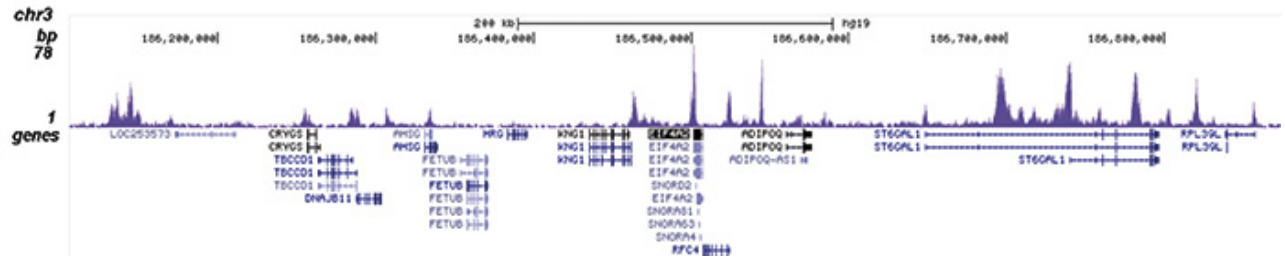

Figure 2. ChIP-seq results obtained with the Diagenode monoclonal antibody directed against TAL1

ChIP was performed with 1 µg of the Diagenode antibody against H3K4ac (Cat. No. C15410322) on sheared chromatin from 4 million HeLa cells as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. 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 peak distribution along the complete sequence and a 1.5 mb region of the X chromosome (figure 2A and B) and in two regions surrounding the GAPDH and EIF4A2 positive control genes (figure 2C and D).

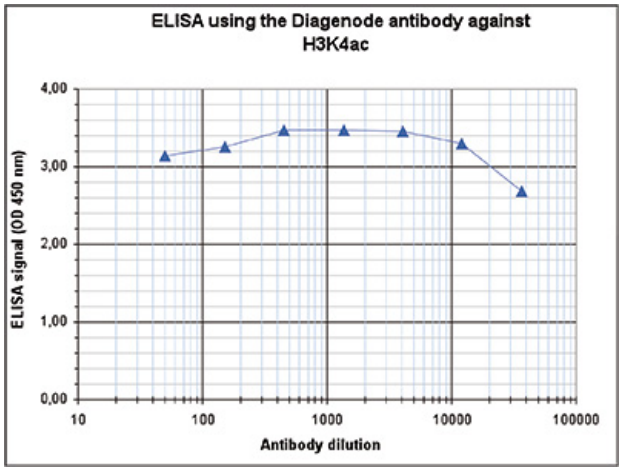

Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K4ac (Cat. No. C15410322) 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 purified antibody was estimated to be 1:197,500.

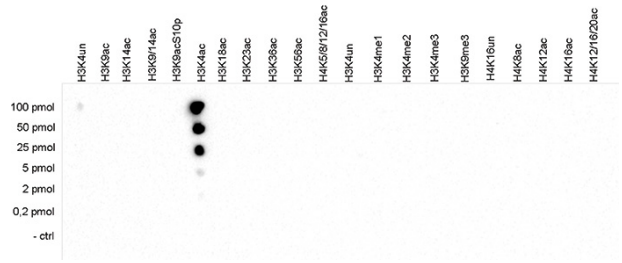

Figure 4. Cross reactivity test using the Diagenode antibody directed against H3K4ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K4ac (Cat. No. C15410322) with peptides containing other histone H3 and H4 modifications and the unmodified H3K4 sequence. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:5,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

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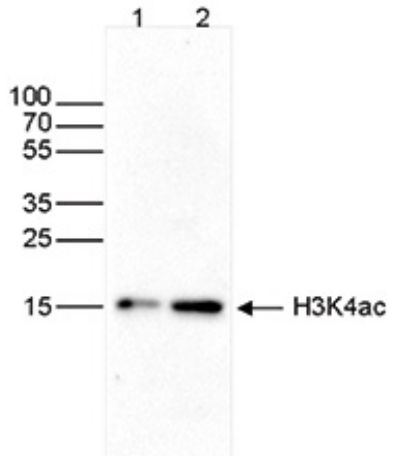

Figure 5. Western blot analysis using the Diagenode antibody directed against H3K4ac

Whole cell (25 ?g, lane 1) and histone extracts (15 µg, lane 2) from HeLa cells were analysed by Western blot using the Diagenode antibody against H3K4ac (Cat. No. C15410322), diluted 1:1,000 in TBSTween containing 5% BSA. The marker (in kDa) is shown on the left, the position of the protein of interest is indicated on the right.

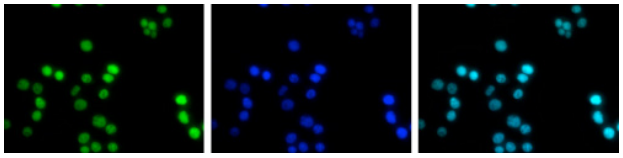

Figure 6. Immunofluorescence using the Diagenode antibody directed against H3K4ac

HeLa cells were stained with the Diagenode antibody against H3K4ac (Cat. No. C15410322) 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 H3K4ac 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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