diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K23ac polyclonal antibody

Cat. No. C15410344

Type: Polyclonal	Specificity: Human: positive. Other species: not tested.
Size: 50 µg	Isotype: NA
Concentration: 2 µg/µl	Source: Rabbit
Lot No.: A2641-0041	Purity: Affinity purified polyclonal antibody in PBS containing 0.05% azide.
Storage buffer: NA	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.	

Last Data Sheet Update: November 21, 2017

Description

Polyclonal antibody raised in rabbit against the region of histone H3 containing the acetylated lysine 23 (H3K23ac), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP *	1 µg/ChIP	Fig 1, 2
ELISA	1:10,000	Fig 3
dot blot	1:20,000	Fig 4
WB	1:1,000	Fig 5
IF	1:1,000	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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Validation data

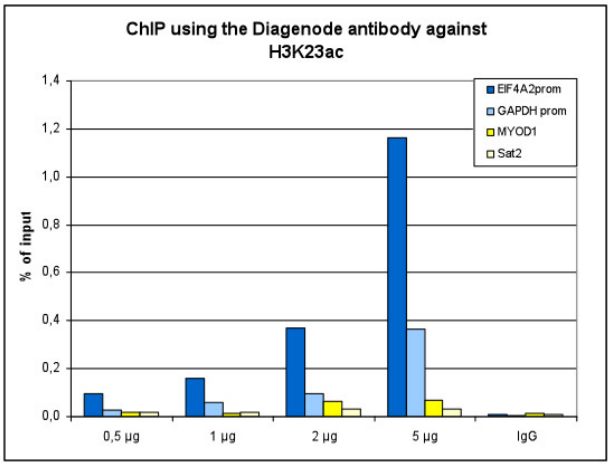
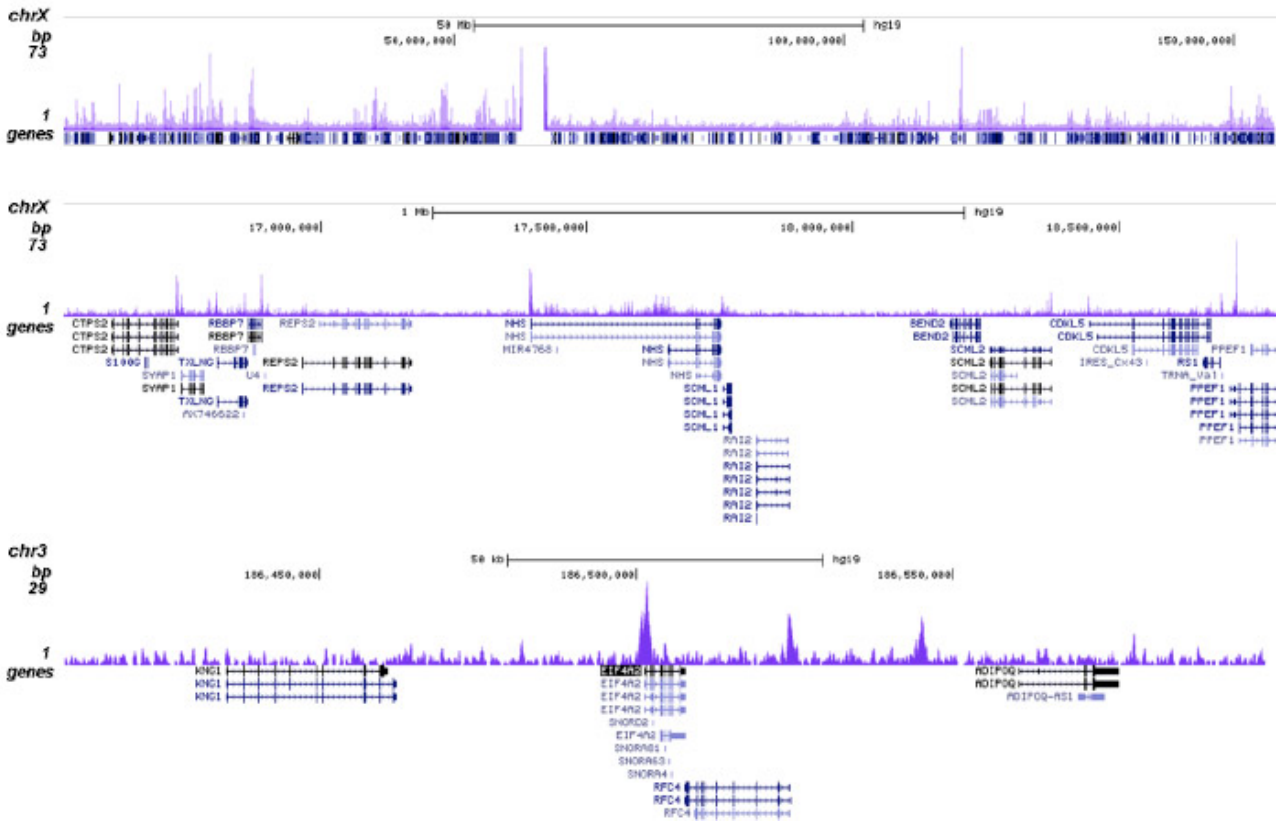


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K23ac

ChIP assays were performed using HeLa cells, the Diagenode antibody against H3K23ac (Cat. No. C15410344) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010055), using sheared chromatin from 1 million cells. A titration consisting of 0.5, 1, 2 and 5 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers for the promoters of the EIF4A2 and GAPDH genes, used as positive controls, and for the MYOD1 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).



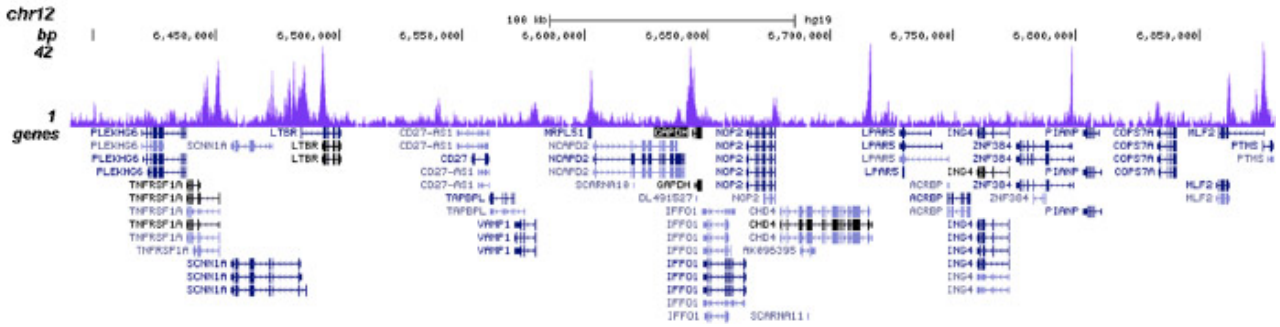


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K23ac

ChIP was performed with 1 µg of the Diagenode antibody against H3K23ac (Cat. No. C15410344) on sheared chromatin from 1,000,000 HeLa cells using the "iDeal ChIP-seq" kit as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 4000. 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 signal distribution along the complete sequence and a 2 Mb region of the human X-chromosome (figures 2A and B), and in two genomic regions surrounding the EIF4A2 and GAPDH positive control genes (figure 2C and D).

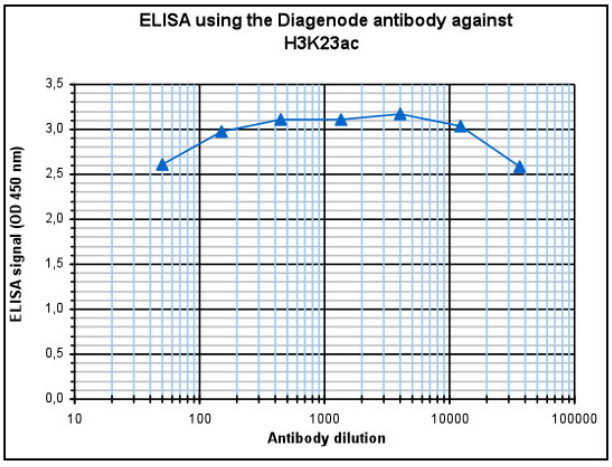


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Diagenode antibody directed against H3K23ac (Cat. No. C15410344). The plates were coated with a peptide containing the modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:1,800,000.

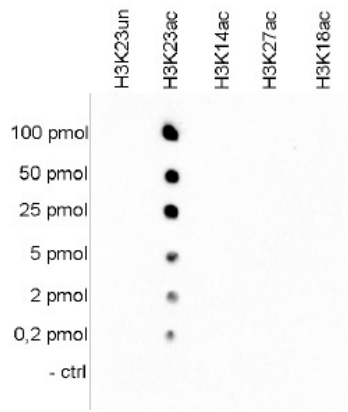


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K23ac

To test the cross reactivity of the Diagenode antibody against H3K23ac (Cat. No. 15410344), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K23. 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 specificity of the antibody for the modification of interest.

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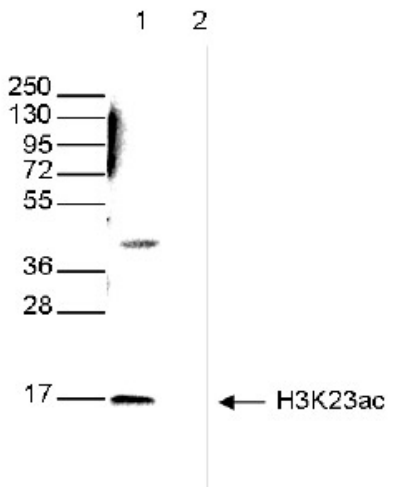
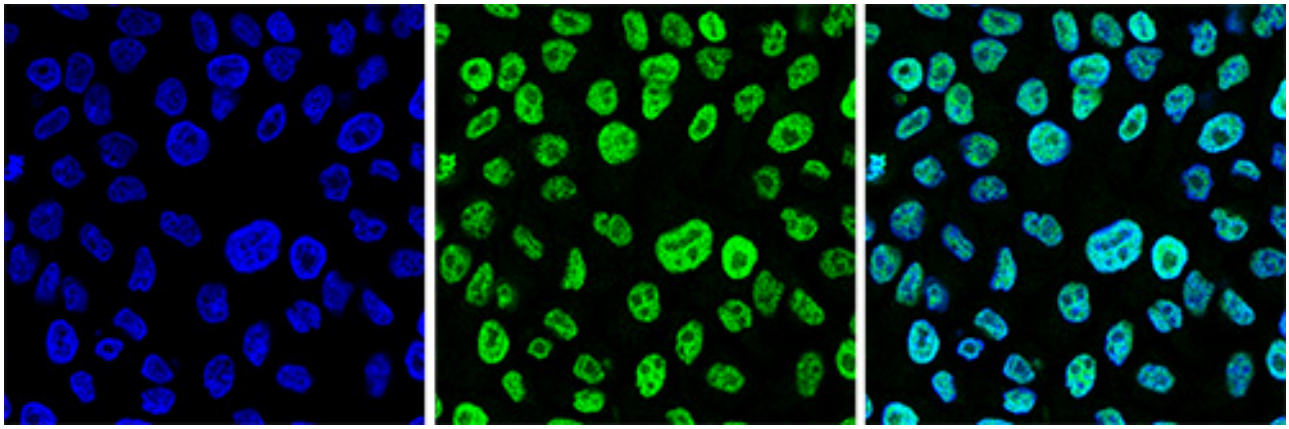


Figure 5. Western blot analysis using the Diagenode antibody directed against H3K23ac

Whole cell extracts from HeLa cells (40 µg, lane 1) and 1 µg of recombinant histone H3 (lane 2) were analysed by Western blot using the Diagenode antibody against H3K23ac (Cat. No. C15410344) diluted 1:1,000 in TBSTween 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.



Immunofluorescence using the Diagenode antibody directed against H3K23ac

HeLa cells were stained with the Diagenode antibody against H3K23ac (Cat. No. C15410344) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labeled with the H3K23ac antibody (middle) diluted 1:1,000 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The left panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right

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