H4K5,8,12,16ac Antibody - ChIP-seq Grade

Cat. No. C15410024

Type: Polyclonal	Specificity: Human, mouse, silena latifolia, wide range expected.
Size: 50 µg	Isotype: NA
Concentration: 0.76 µg/µl	Host: Rabbit
Lot No.: A0607P	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.	

Last Data Sheet Update: June 17, 2020

Description

Polyclonal antibody raised in rabbit against the region of histone **H4 containing the acetylated lysines 5, 8, 12 and 16 (H4K5,8,12,16ac)**, using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	2 µg/IP	Fig 1, 2
ELISA	1:1,000	Fig 3
Dot Blotting	1:20,000	Fig 4
IF	1:500	Fig 5

* Please note that the optimal antibody amount per ChIP 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. Acetylation of histone H4 is associated with active genes.

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Validation data

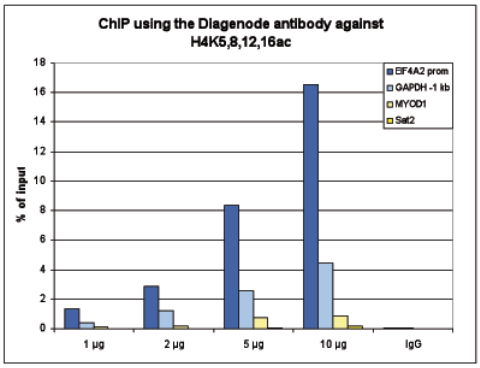
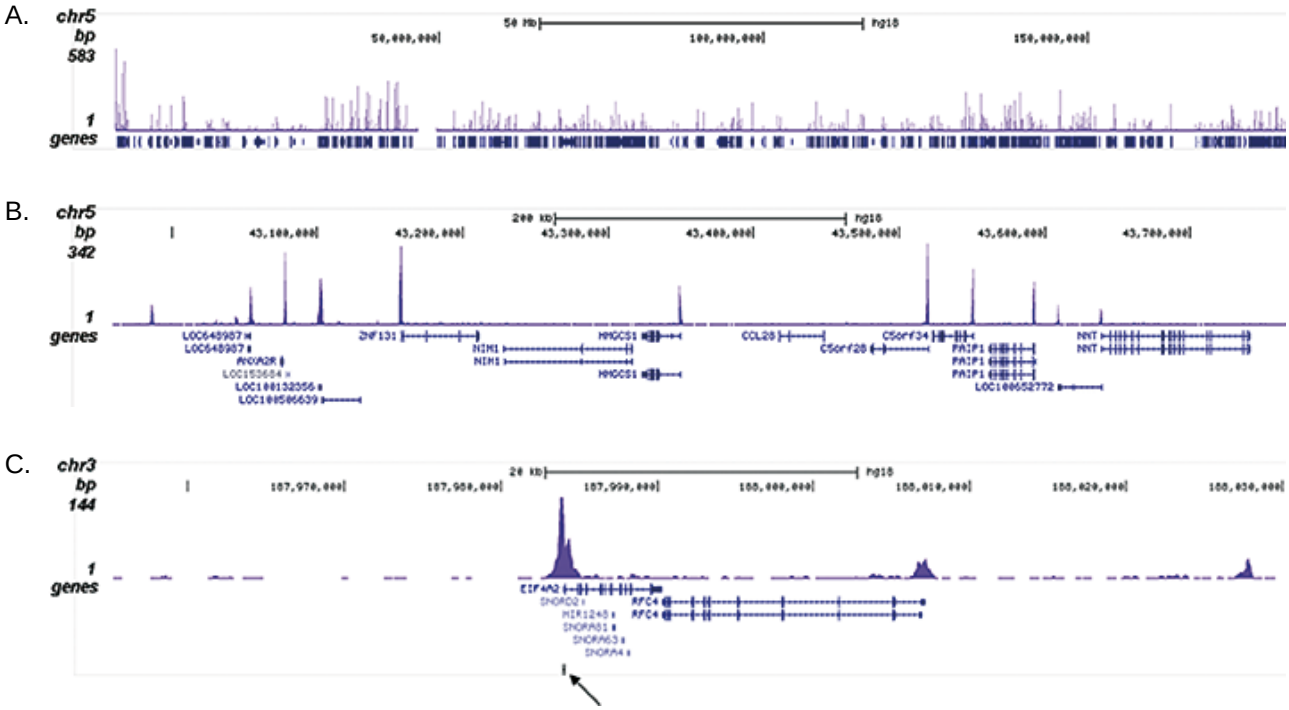


Figure 1. ChIP results obtained with the Diagenode antibody directed against H4K5,8,12,16ac

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H4K5,8,12,16ac (Cat. No. C15410024) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP- seq" kit (Cat. No. AB-001-0024) on 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 promoter of the active gene EIF4A2 and for a region 1 kb upstream of the GAPDH gene, used as positive controls, and for the inactive MYOD1 gene and the Sat2 satellite repeat region 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).



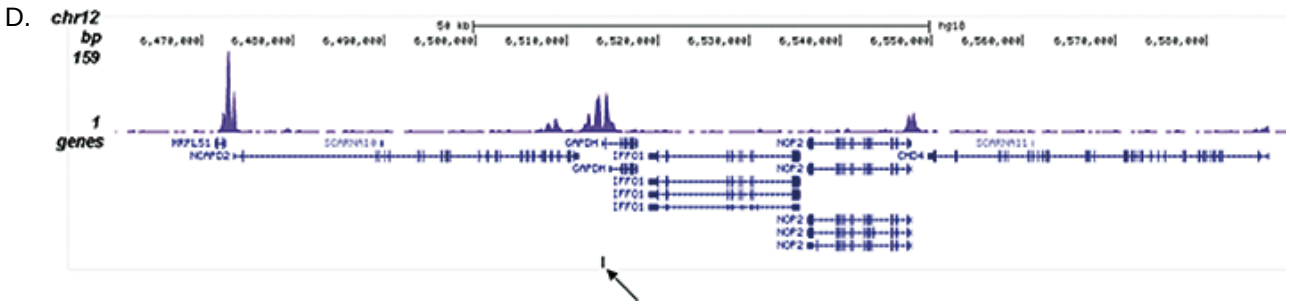


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H4K5,8,12,16ac

ChIP was performed with 2 ?g of the Diagenode antibody against H4K5,8,12,16ac (Cat. No. C15410024) on sheared chromatin from 1 million HeLa cells using the "iDeal ChIP-seq" kit as described above. 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 signal distribution along the complete length of chromosome 5 (figure 2A) and a zoomin to a 600 kb region (figure 2B). Figure 2C and D show the enrichment in two genomic regions on chromosome 3 and 12, respectively, containing EIF4A2 and GAPDH positive controls. The position of the amplicon used for validating the QPCR results is shown with an arrow

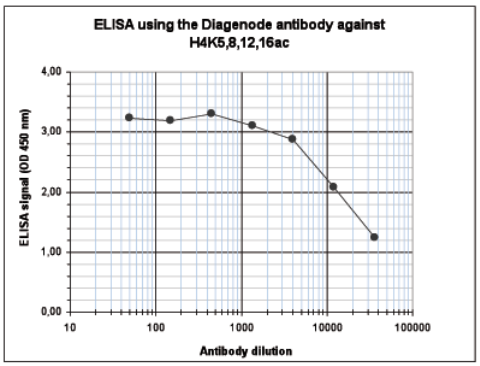


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H4K5,8,12,16ac (Cat. No. C15410024) 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 1:21,200.

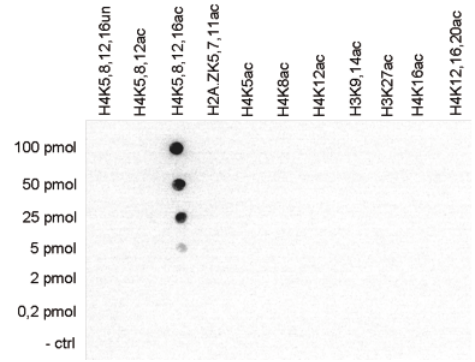


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H4K5,8,12,16ac

To test the cross reactivity of the Diagenode antibody against H4K5,8,12,16ac (Cat. No. C15410024), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H4. 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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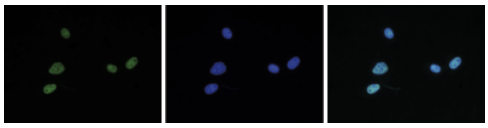

Figure 5. Immunofluorescence using the Diagenode antibody directed against H4K5,8,12,16ac

Mouse NIH3T3 cells were stained with the Diagenode antibody against H4K5,8,12,16ac (Cat. No. C15410024) 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 H4K5,8,12,16ac 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.

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