

H4K5ac antibody

Cat. No. C15410025

Type: Polyclonal ChIP grade/ChIP-seq grade	Specificity: Human, mouse, wide range expected.
Source: Rabbit	Purity: Affinity purified polyclonal antibody.
Lot: A1456D	Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Size: 50 µg	Storage buffer: PBS containing 0.05% azide and 0.05% ProClin 300.
Concentration: 1.80 µg/µl	

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Polyclonal antibody raised in rabbit against the region of histone H4 containing the acetylated lysine 5 (H4K5ac), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	1 - 2 µg per IP	Fig 1, 2
CUT&TAG	1 µg	Fig 3
ELISA	1:100 - 1:500	Fig 4
Dot blotting	1:5,000	Fig 5
Western blotting	1:500	Fig 6
Immunofluorescence	1:500	Fig 7

*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. Acetylation of histone H4 is associated with active genes.

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Results

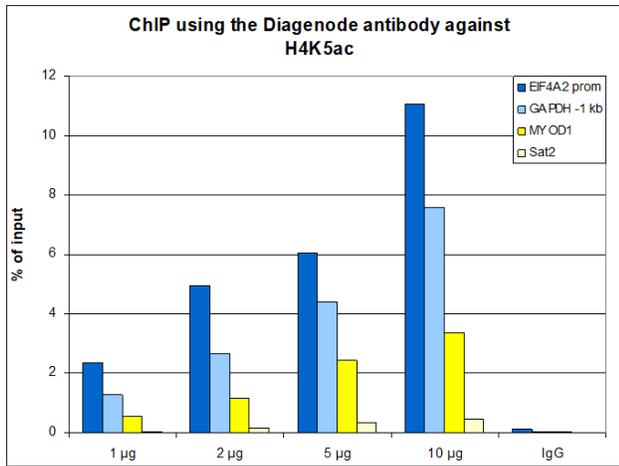


Figure 1. ChIP results obtained with the Diagenode antibody directed against H4K5ac

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H4K5ac (cat. No. C15410025) and optimized PCR primer sets for qPCR. ChIP was performed with the “iDeal ChIP-seq” kit (cat. No. C01010051) 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).

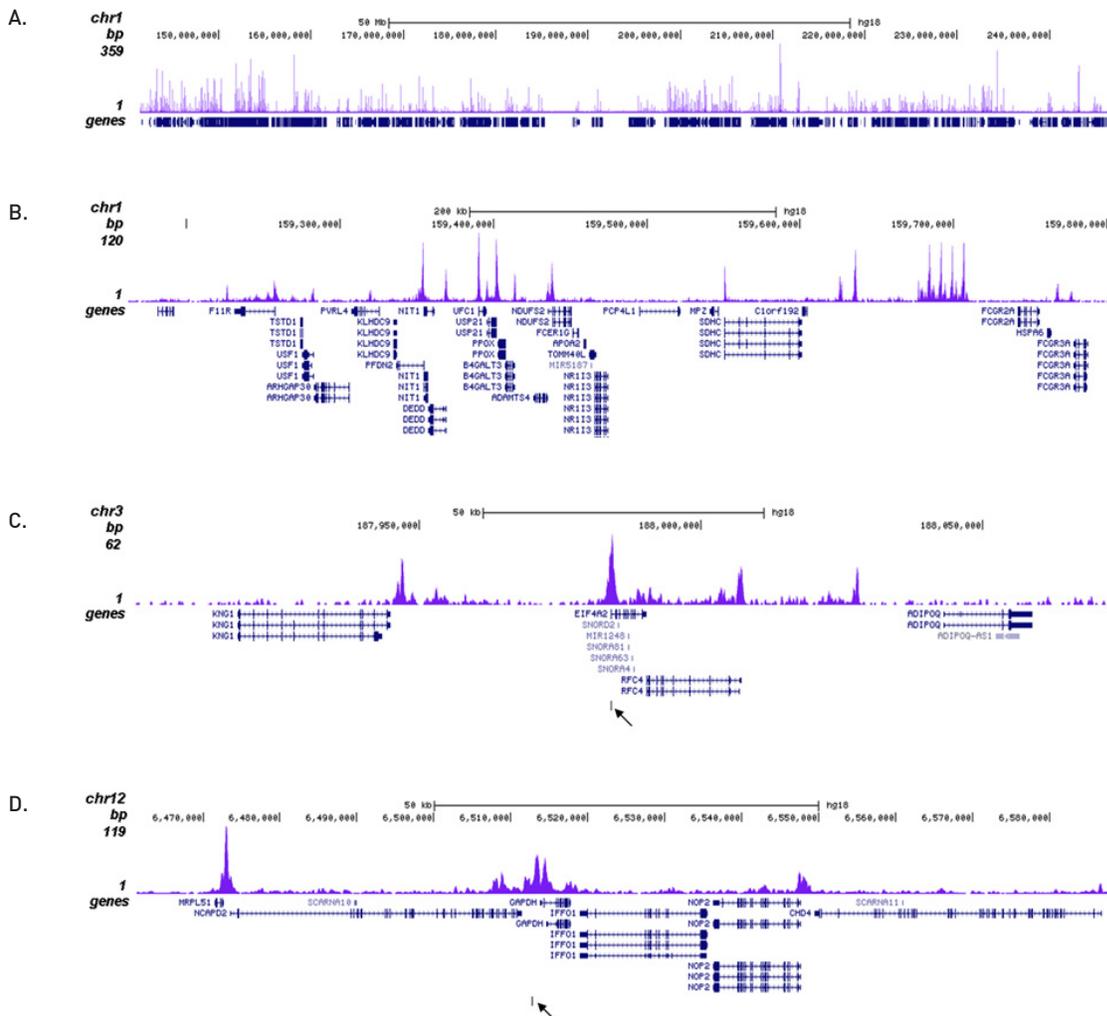


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H4K5ac

ChIP was performed with 1 µg of the Diagenode antibody against H4K5ac (cat. No. C15410025) 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 1 (figure 2A) and a zoomin to a 500 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

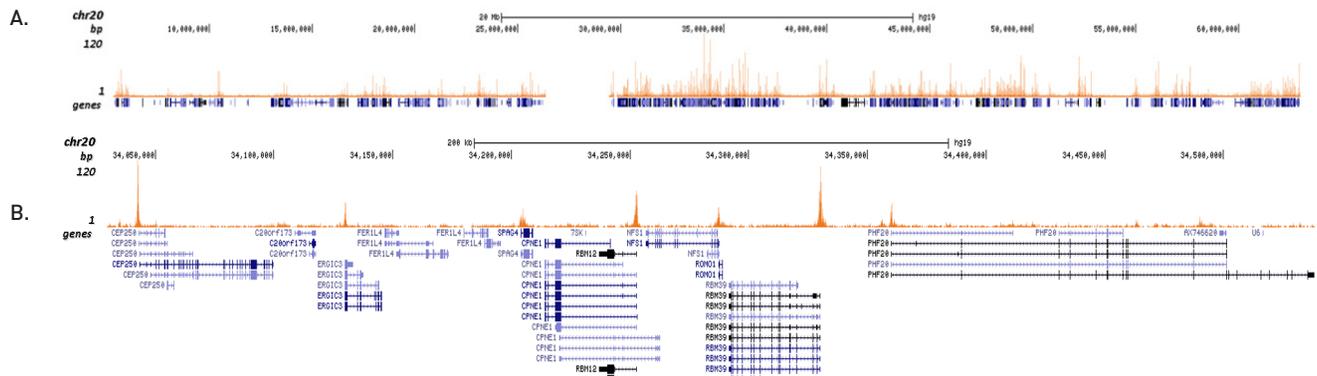


Figure 3. Cut&Tag results obtained with the Diagenode antibody directed against H4K5ac

CUT&TAG (Kaya-Okur, H.S., Nat Commun 10, 1930, 2019) was performed on 50,000 K562 cells using 1 µg of the Diagenode antibody against H4K5ac (cat. No. C15410025) and the Diagenode pA-Tn5 transposase (C01070001). The libraries were subsequently analysed on an Illumina NextSeq 500 sequencer (2x75 bp paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg19) using the BWA algorithm. Figure 3 shows the peak distribution along the complete sequence and a 1.5 Mb zoomin of chromosome 20 (figure 3A and B, respectively).

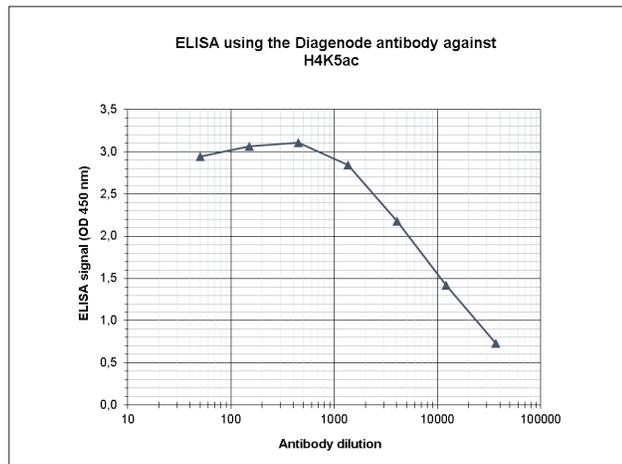


Figure 4. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H4K5ac (cat. No. C15410025) 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 4), the titer of the antibody was estimated to be 1:9,900.

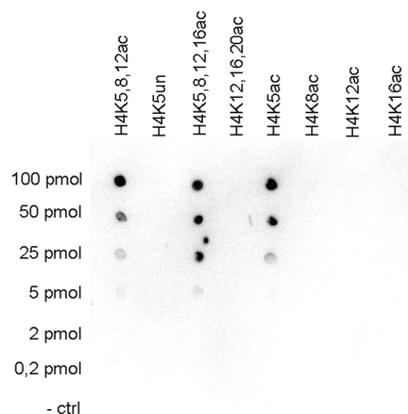


Figure 5. Cross reactivity tests using the Diagenode antibody directed against H4K5ac

To test the cross reactivity of the Diagenode antibody against H4K5ac (cat. No. C15410025), 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 1:5,000. Figure 5 shows a high specificity of the antibody for the modification of interest.

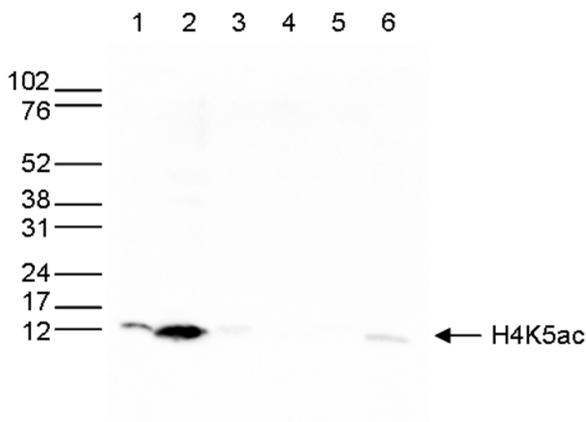


Figure 6. Western blot analysis using the Diagenode antibody directed against H4K5ac

Western blot was performed on whole cell (25 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 H4K5ac (cat. No. C15410025). The antibody was diluted 1:500 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.

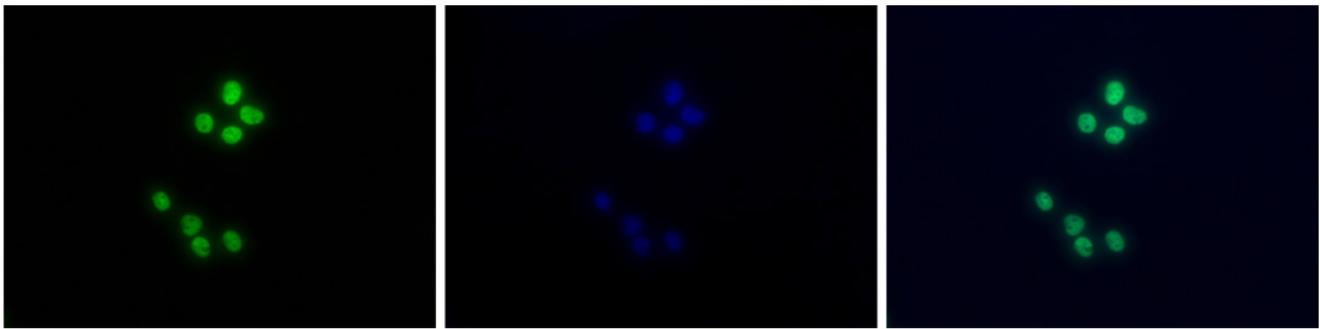


Figure 7. Immunofluorescence using the Diagenode antibody directed against H4K5ac

HeLa cells were stained with the Diagenode antibody against H4K5ac (cat. C15410025) 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 H4K5ac 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.