diagenode
Innovating Epigenetics Solutions

TECHNICAL DATASHEET

H3K27ac Antibody - ChIP-seq Grade

Cat. No. C15410196

Type: Polyclonal, **ChIP grade, ChIP-seq grade**	Specificity: Human, mouse, rat, Arabidopsis, wide range expected
Size: 50 µg	Isotype: NA
Concentration: 2.8 µg/µl	Host: Rabbit
Lot No.: A1723-0041D	Purity: Affinity purified
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: January 11, 2021

Description

Polyclonal antibody raised in rabbit against the region of histone **H3 containing the acetylated lysine 27** (**H3K27ac**), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	1 µg/IP	Fig 1, 2
CUT&TAG	1 µg	Fig 3
ELISA	1:500	Fig 4
Dot Blotting	1:20,000	Fig 5
Western Blotting	1:1,000	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 0.5-5 µg per IP.

Target Description

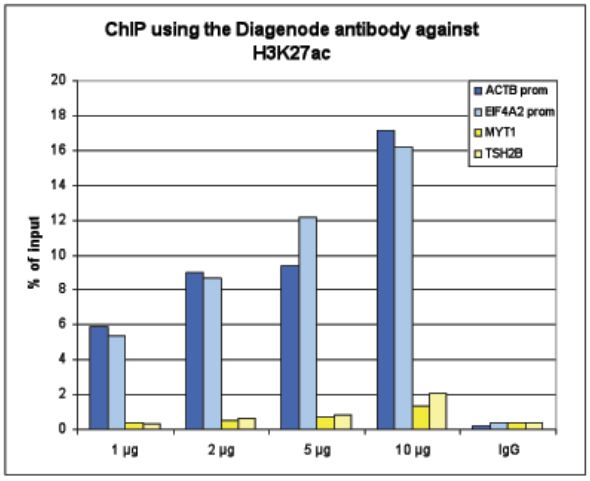
Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble 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 H3K27 is associated with active promoters and enhancers.

Diagenode sa. BELIGUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
info@diagenode.com | orders@diagenode.com

Diagenode Inc. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
info.na@diagenode.com | orders.na@diagenode.com

Validation Data

A.



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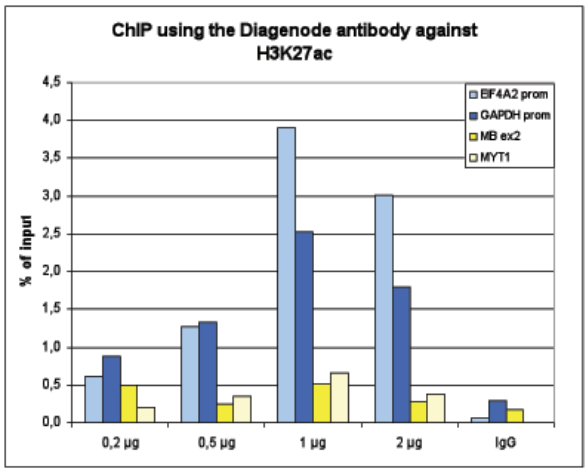

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K27ac

Figure 1A ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K27ac (Cat. No. C15410196) and optimized PCR primer pairs for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit on the IP-Star automated system, using sheared chromatin from 1,000,000 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 for the promoters of the active EIF4A2 and ACTB genes, used as positive controls, and for the inactive TSH2B and MYT1 genes, used as negative controls.

Figure 1B ChIP assays were performed using human K562 cells, the Diagenode antibody against H3K27ac (Cat. No. C15410196)and optimized PCR primer pairs for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), using sheared chromatin from 100,000 cells. A titration consisting of 0.2, 0.5, 1 and 2 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 active GAPDH and EIF4A2 genes, used as positive controls, and for the coding regions of the inactive MB and MYT1 genes, 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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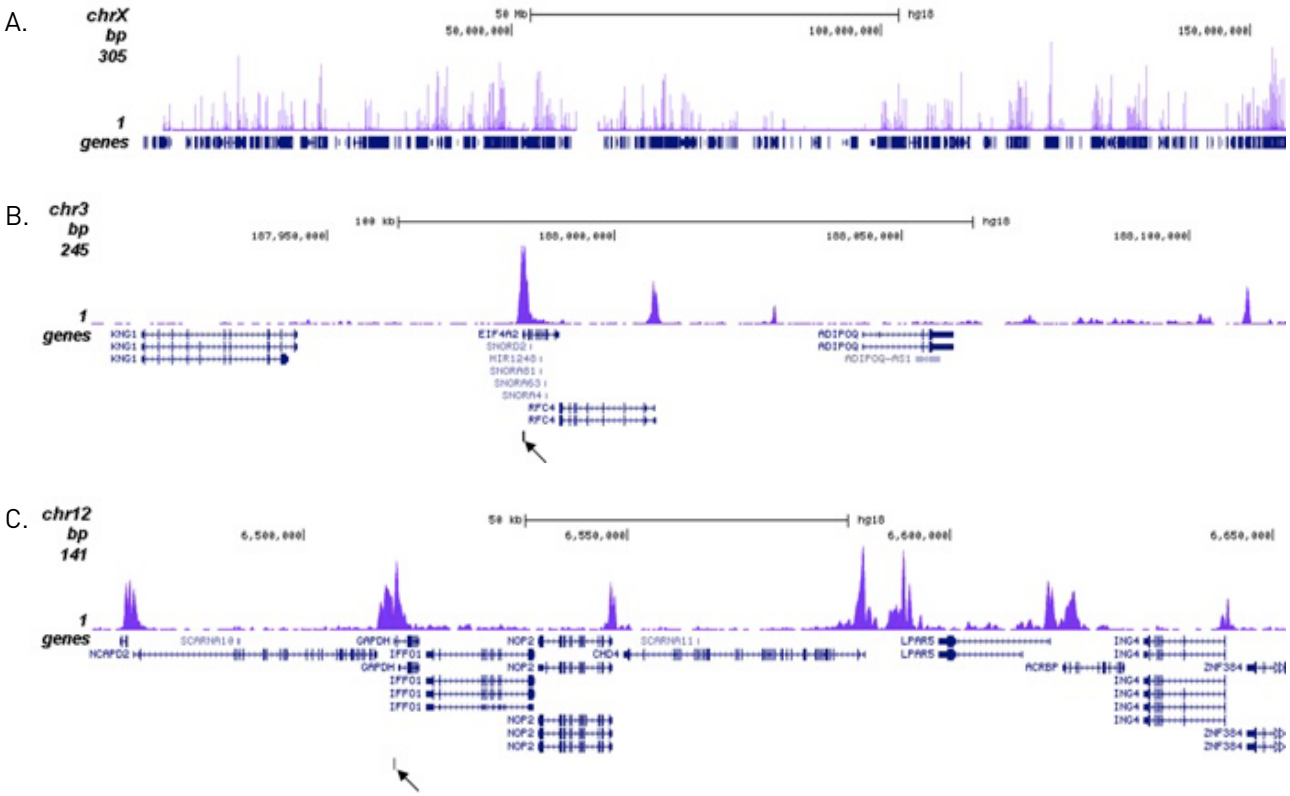

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K27ac

ChIP was performed on sheared chromatin from 100,000 K562 cells using 1 µg of the Diagenode antibody against H3K27ac (Cat. No. C15410196) 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 2A shows the peak distribution along the complete human X-chromosome. Figure 2 B and C show the peak distribution in two regions surrounding the EIF4A2 and GAPDH positive control genes, respectively. The position of the PCR amplicon, used for validating the ChIP assay is indicated with an arrow.

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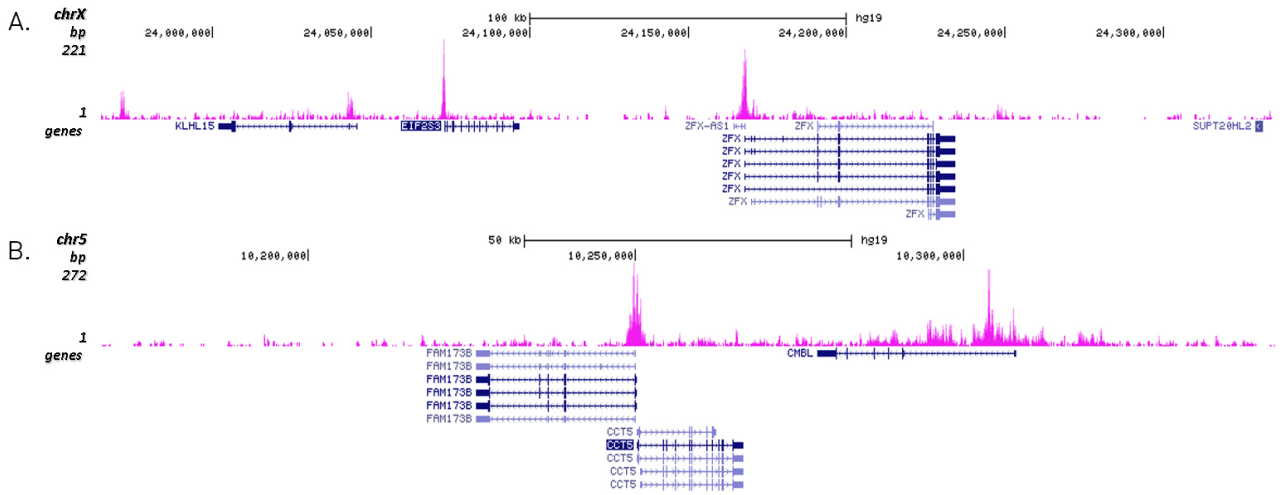

Figure 3. Cut&Tag results obtained with the Diagenode antibody directed against H3K27ac

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 H3K27ac (cat. No. C15410196) and the Diagenode pA-Tn5 transposase (C01070001). The libraries were subsequently analysed on an Illumina NextSeq 500 sequencer (2x75 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 in 2 genomic regions surrounding the EIF2S3 gene on the X-chromosome and the CCT5 gene on chromosome 5 (figure 3A and B, respectively).

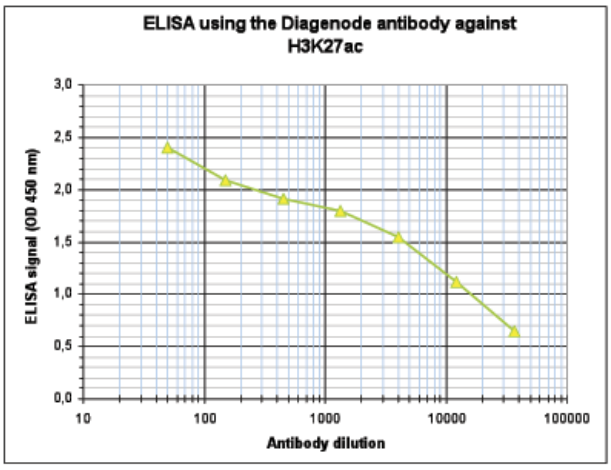

Figure 4. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against H3K27ac (Cat. No. C15410196). 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 1:8,300.

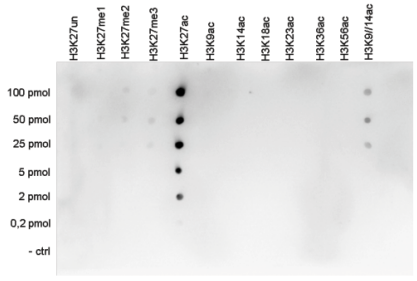

Figure 5. Cross reactivity tests using the Diagenode antibody directed against H3K27ac

To test the cross reactivity of the Diagenode antibody against H3K27ac (Cat. No. C15410196), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K27. 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 5 shows a high specificity of the antibody for the modification of interest.

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LIEGE SCIENCE PARK
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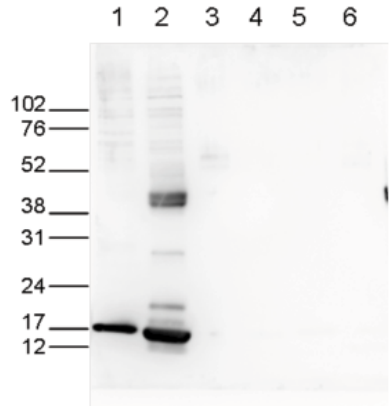

Figure 6. Western blot analysis using the Diagenode antibody directed against H3K27ac

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 H3K27ac (Cat. No. C1541196). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.

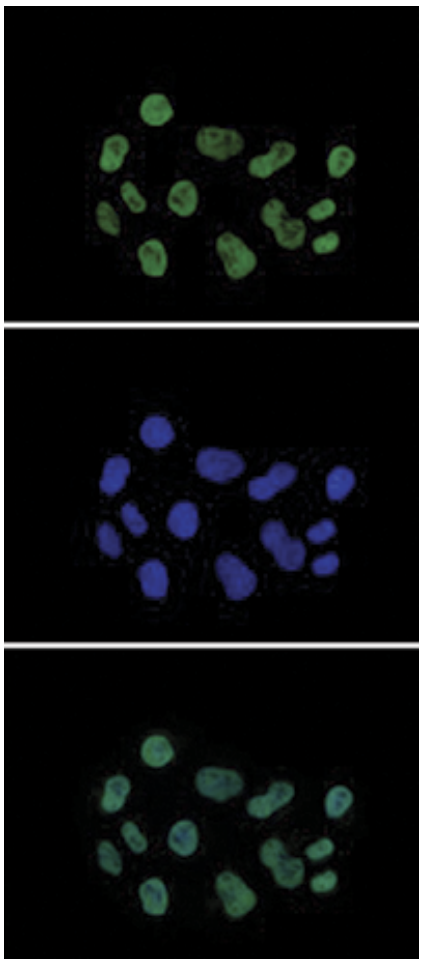

Figure 7. Immunofluorescence using the Diagenode antibody directed against H3K27ac

HeLa cells were stained with the Diagenode antibody against H3K27ac (Cat. No. C15410196) 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 H3K27ac antibody (top) 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 at the bottom.

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