

## H3K56ac antibody

**Cat. No.** C15410213

**Type:** Polyclonal, ChIP grade/ChIP-seq grade

**Source:** Rabbit

**Lot:** A2069P

**Size:** 50 µg

**Concentration:** 0.8 µg/µl

**Specificity:** Human: positive  
Other species: not tested.

**Purity:** Affinity purified polyclonal antibody.

**Storage:** Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

**Storage buffer:** PBS containing 0.05% azide and 0.05% ProClin 300.

**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 H3 containing the acetylated lysine 56 (H3K56ac), using a KLH-conjugated synthetic peptide.

### Applications

| Applications     | Suggested dilution | References |
|------------------|--------------------|------------|
| ChIP*            | 1 µg per ChIP      | Fig 1, 2   |
| CUT&TAG          | 1 µg               | Fig 3      |
| ELISA            | 1:1000             | Fig 4      |
| Dot Blotting     | 1:20,000           | Fig 5      |
| Western Blotting | 1:200              | 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.

#### Diagenode sa. BELGIUM | EUROPE

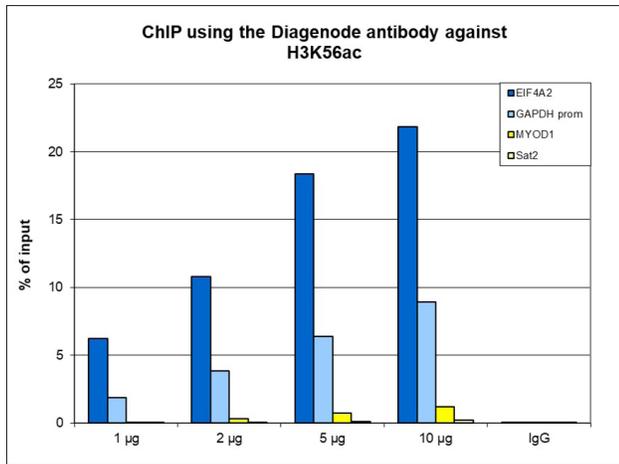
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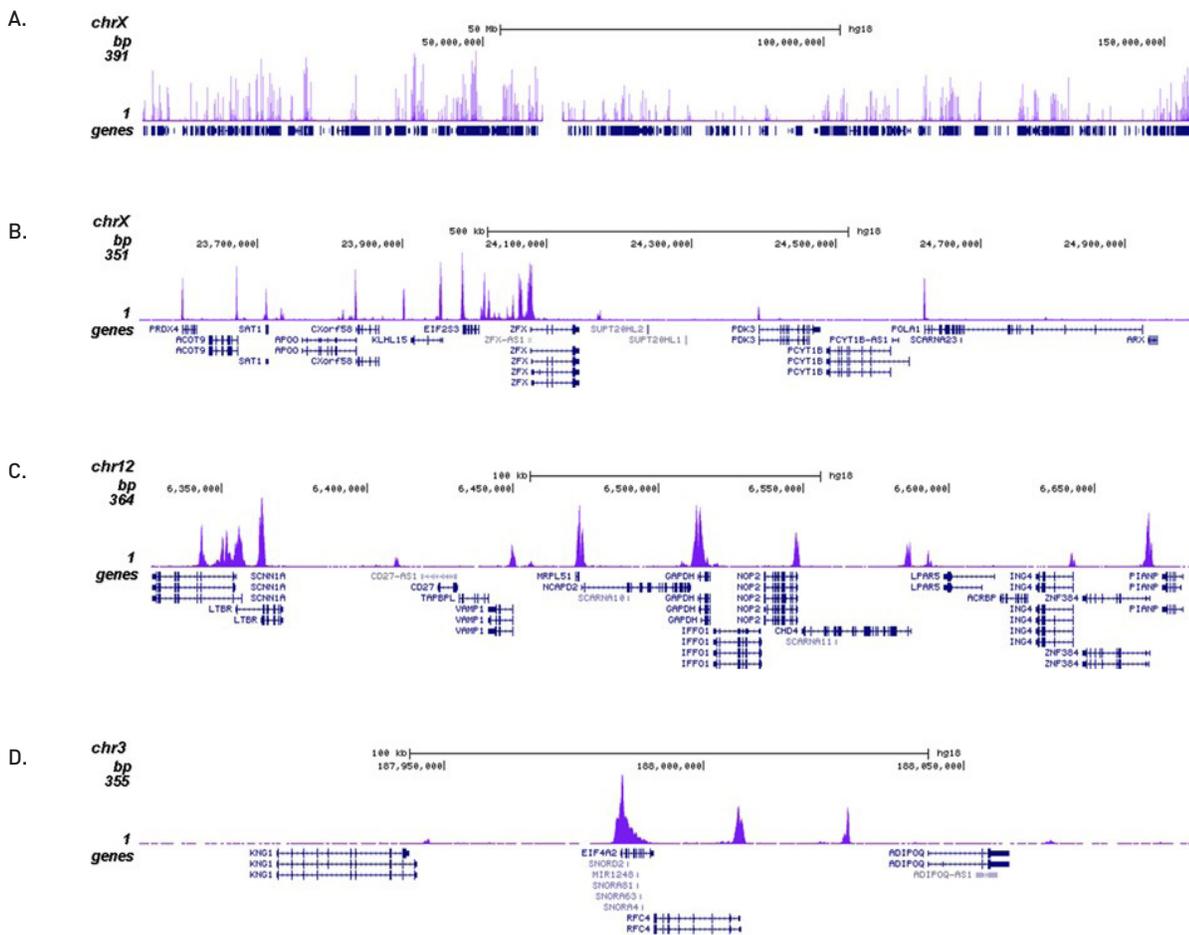
Last update: February, 2023

## Results



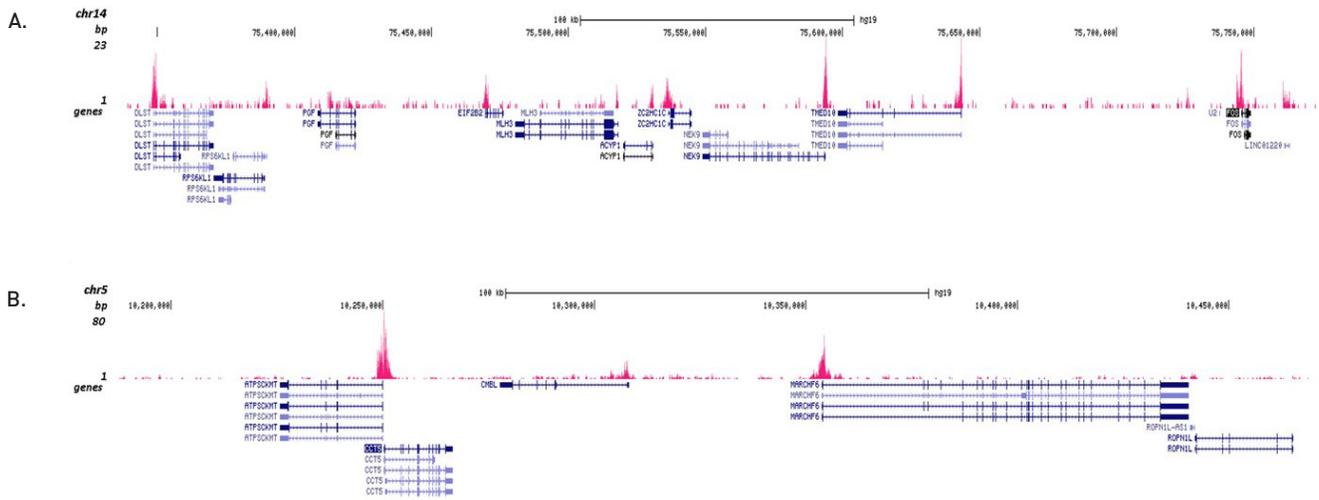
**Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K56ac**

ChIP assays were performed using human K562 cells, the Diagenode antibody against H3K56ac (cat. No. C15410213) and optimized PCR primer sets for qPCR. ChIP was performed with the “iDeal ChIP-seq” kit (cat. No. C01010051), using sheared chromatin from 500,000 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 GAPDH and EIF4A2 promoters, used as positive controls, and for the coding region of the inactive 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).



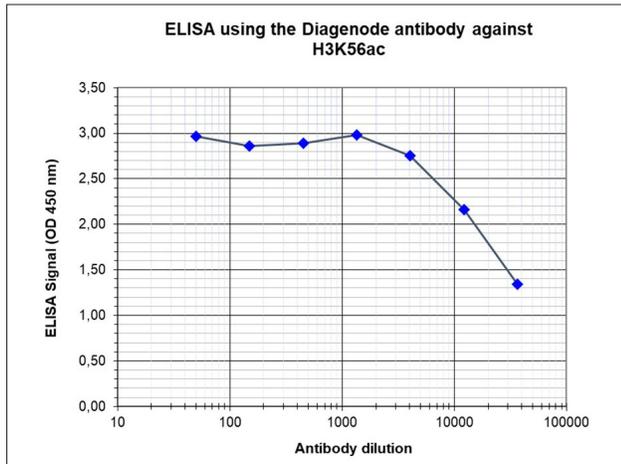
**Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K56ac**

ChIP was performed on sheared chromatin from 500,000 K562 cells using 1 µg of the Diagenode antibody against H3K56ac (cat. No. C15410213) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment along the complete sequence and a 1 Mb region of the X-chromosome (fig 2A and B) and in genomic regions of chromosome 12 and 3, surrounding the GAPDH and EIF4A2 positive control genes.



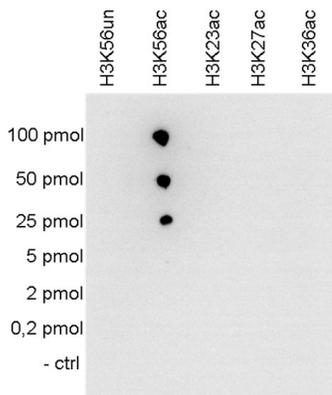
**Figure 3. Cut&Tag results obtained with the Diagenode antibody directed against H3K56ac**

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 H3K56ac (cat. No. C15410213) 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 FOS gene on chromosome 14 and the CCT5 gene on chromosome 5 (figure 3A and B, respectively).



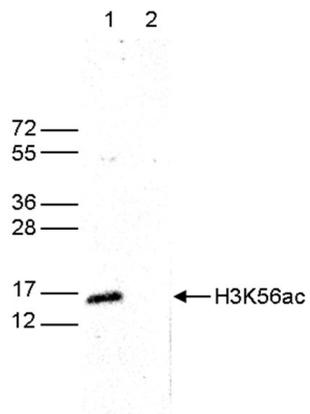
**Figure 4. Determination of the antibody titer**

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K56ac (cat. No. 15410213) 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 1:36,500.



**Figure 5. Cross reactivity tests using the Diagenode antibody directed against H3K56ac**

To test the cross reactivity of the Diagenode antibody against H3K56ac (cat. No. 15410213), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K56. 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.



**Figure 6. Western blot analysis using the Diagenode antibody directed against H3K56ac**

Western blot was performed on whole cell extracts (40  $\mu$ g, lane 1) from HeLa cells, and on 1  $\mu$ g of recombinant histone H3 (lane 2) using the Diagenode antibody against H3K56ac (cat. No. C15410213). The antibody was diluted 1:200 in TBS-Tween 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.