diagenode
A Hologic Company

TECHNICAL DATASHEET

# H2A.Zac Antibody - ChIP-seq Grade

## Cat. No. C15410173

| | |
|---|---|
| Type: Polyclonal, **ChIP grade, ChIP-seq grade** | Specificity: Human: positive. Other species: not tested. |
| Size: 50 µg | Isotype: NA |
| Concentration: 1.4 µg/µl | Host: Rabbit |
| Lot No.: A1775P | 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: February 10, 2021

## Description

Polyclonal antibody raised in rabbit against histone **H2A.Z acetylated at lysines 4, 7 and 11,** using a KLH-conjugated synthetic peptide.

## Applications

| Applications | Suggested dilution | References |
|---|---|---|
| ChIP/ChIP-seq * | 0.5 µg/ChIP | Fig 1, 2 |
| ELISA | 1:5,000 | Fig 3 |
| Dot Blotting | 1:20,000 | Fig 4 |
| Western Blotting | 1:1,000 | Fig 5 |
| Immunofluorescence | 1:500 | Fig 6 |

* 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 the histone H2A variant H2A.Z is associated with the promoters of active genes.

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## Validation Data

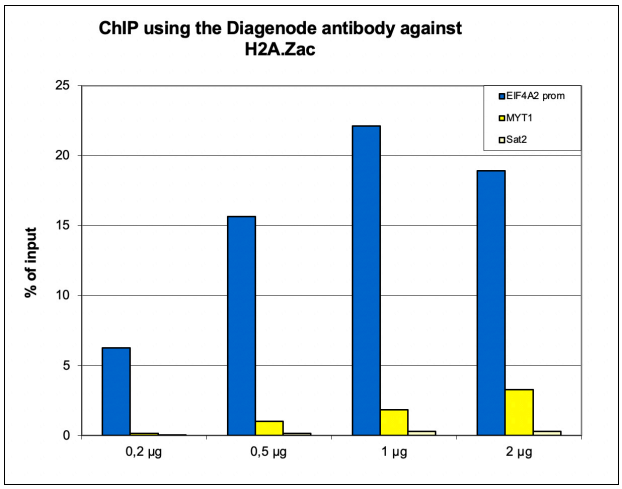
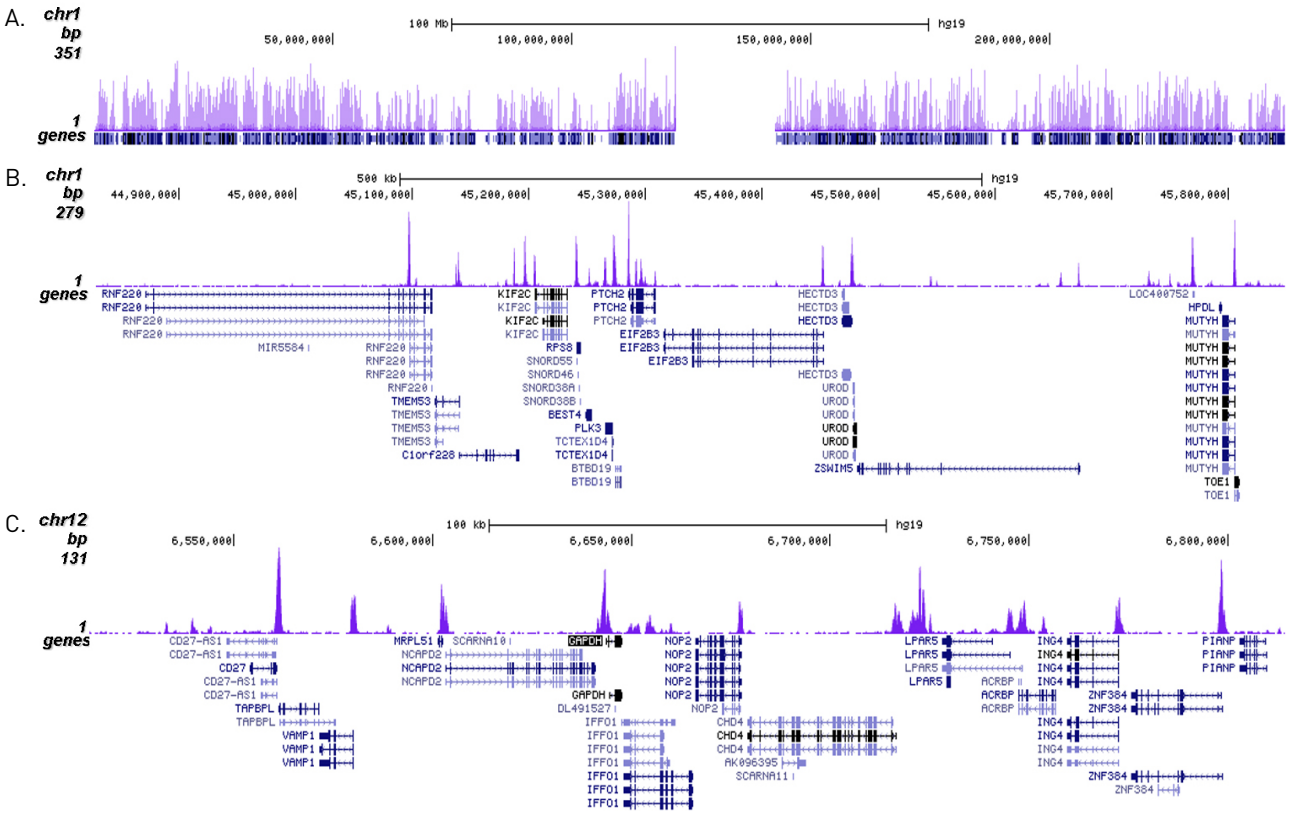



**Figure 1. ChIP results obtained with the Diagenode antibody directed against H2A.Zac**

ChIP assays were performed using HeLa cells, the Diagenode antibody against H2A.Zac (cat. No. C15410173) and optimized primer pairs for qPCR. ChIP was performed on sheared chromatin from 100,000 K562 cells using the iDeal ChIP-seq kit. A titration of the antibody consisting of 0.2, 0.5, 1 and 2 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed using primers specific for the promoter of the EIF4A2 gene, used as positive control target and for the coding region of the MYT1 gene, and the Sat2 satellite repeat, used as negative control targets. Figure 1 shows the recovery (the relative amount of immunoprecipitated DNA compared to input DNA).

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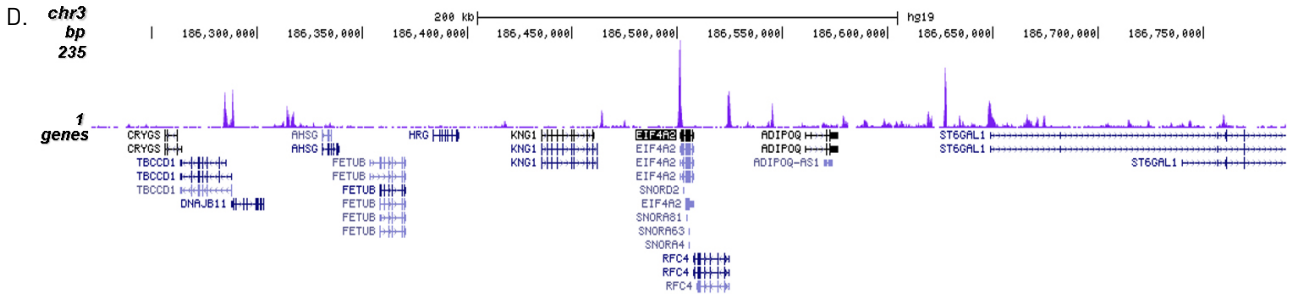


**Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H2A.Zac**

ChIP was performed with 0.5 µg of the Diagenode antibody against H2A.Zac (cat. No. C15410173) as described above. The IP'd DNA was subsequently analysed with 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 peak distribution along the complete sequence and a 1 Mb region of human chromosome 1 (figure 2A and B) and in two regions surrounding the GAPDH and the EIF4A2 positive control gene (figure 2C and D, respectively).

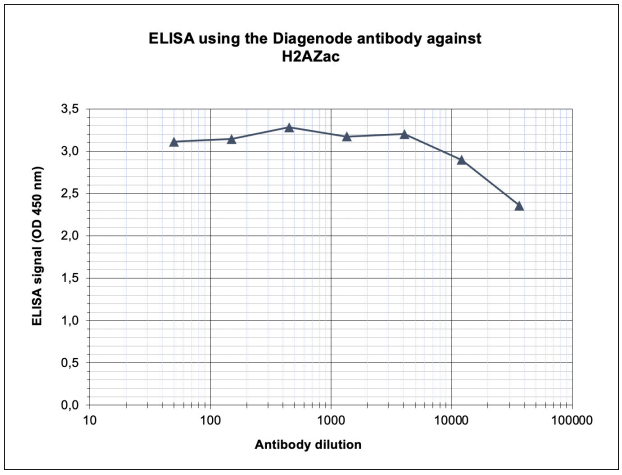


**Figure 3. Determination of the antibody titer**

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H2A.Zac (cat. No. C15410173). The antigen used was a peptide containing the histone modifications of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the purified antibody was estimated to be 1:265,000.

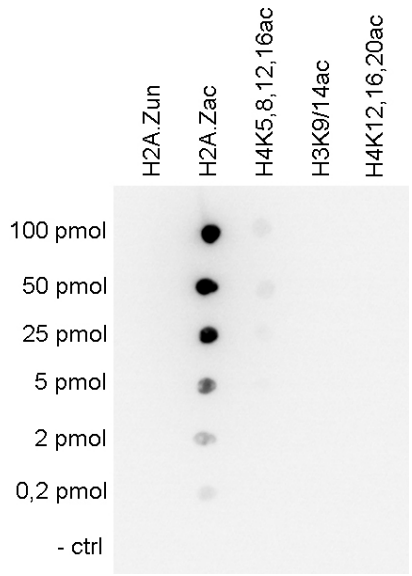


**Figure 4. Cross reactivity test using the Diagenode antibody directed against H2A.Zac**

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H2A.Zac (cat. No. C15410173) with peptides containing other histone acetylations and the unmodified H2A.Z sequence. 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.

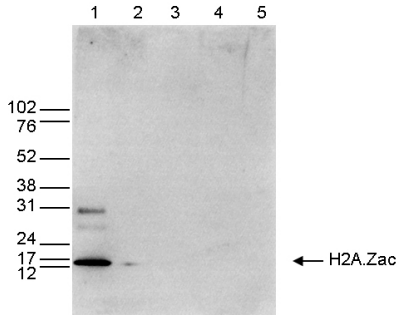


**Figure 5. Western blot analysis using the Diagenode antibody directed against H2A.Zac**

Western blot was performed on whole cell extracts (25 µg, lane 1) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 2, 3, 4 and 5, respectively) using the Diagenode antibody against H2A.Zac (cat. No. C15410173). The antibody was diluted 1:1,000 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.

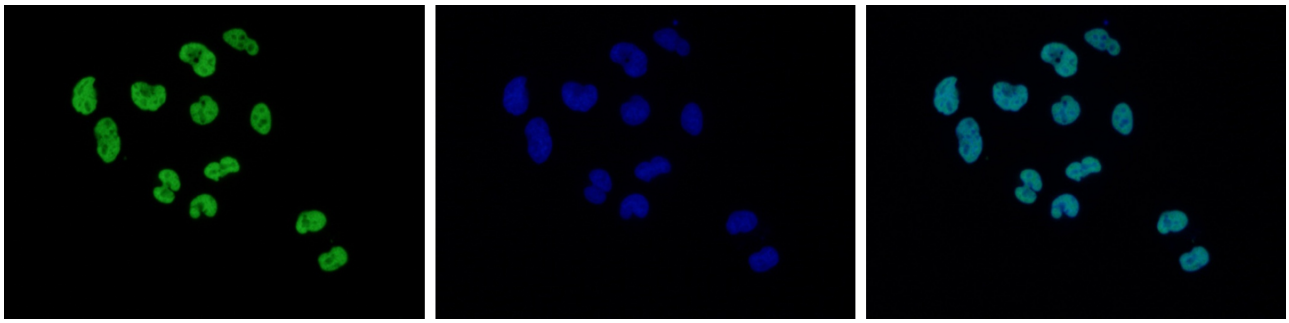

**Figure 6. Immunofluorescence using the Diagenode antibody directed against H2A.Zac**

HeLa cells were stained with the Diagenode antibody against H2A.Zac (cat. No. C15410173) 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 labelled with the H2A.Zac 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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