

H2A.Zac polyclonal antibody

Cat. No. C15410202

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

Source: Rabbit

Lot #: A1738P

Size: 50 µg/ 46 µl

Concentration: 1.09 µg/µl

Specificity: Human, mouse, wide range expected.

Purity: Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.

Storage: 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.

Description: Polyclonal antibody raised in rabbit against the region of histone H2A.Z containing the acetylated lysines 4, 7 and 11, using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP*	1 µg per IP	Fig 1, 2
ELISA	1:500	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.

Results

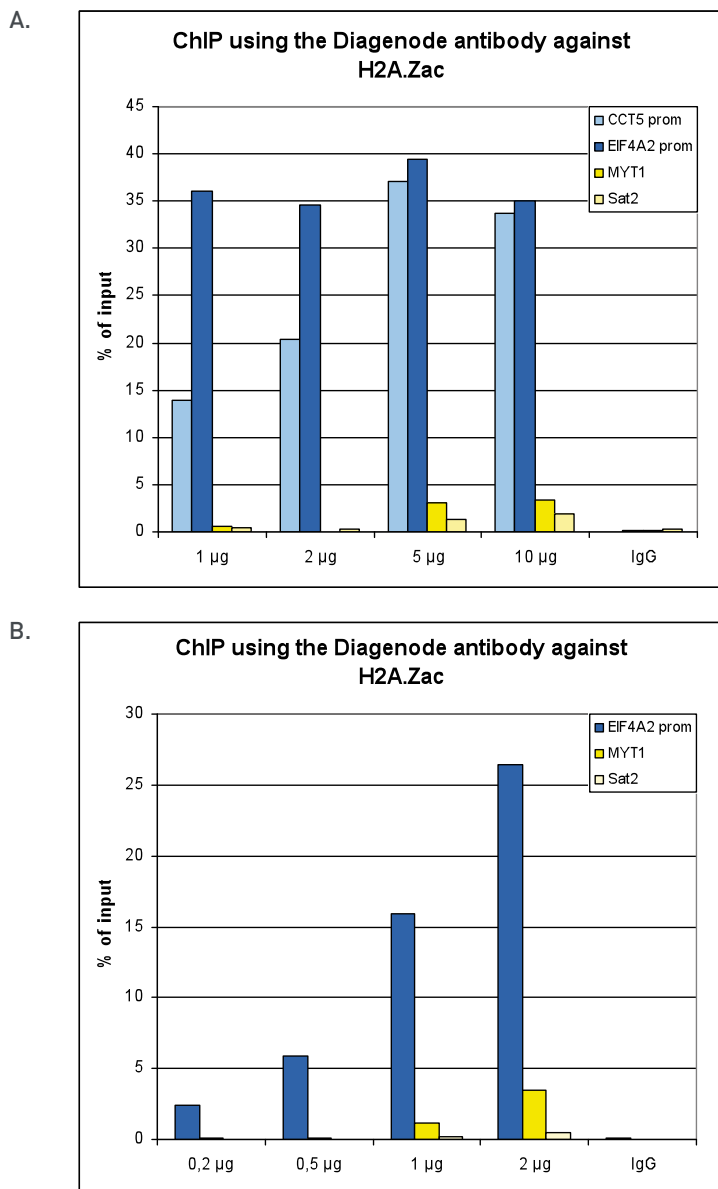


Figure 1. ChIP results obtained with the Diagenode antibody directed against H2A.Zac

Figure 1A ChIP assays were performed using human HeLa cells, the Diagenode antibody against H2A.Zac (Cat. No. C15410202) and optimized PCR primer pairs for qPCR. ChIP was performed with the “Auto Histone ChIP-seq” kit (Cat. No. AB-Auto02-A100) 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.

Figure 1B ChIP assays were performed using human K562 cells, the Diagenode antibody against H2A.Zac (Cat. No. C15410202) 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 100,000 cells. 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.

Quantitative PCR was performed with primers specific for the promoter of the active genes CCT5 and EIF4A2, used as positive controls, and for the coding region of the inactive MYT1 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).

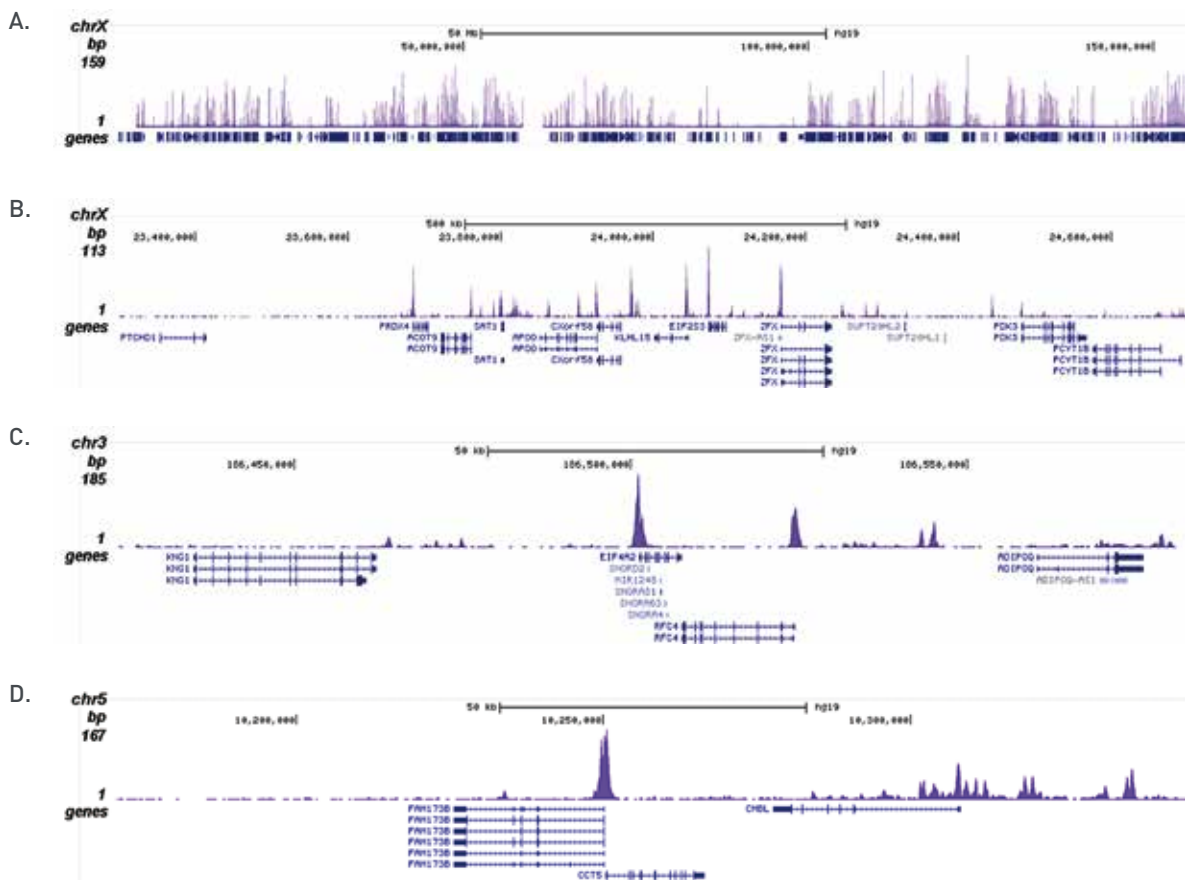


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H2A.Zac

ChIP was performed on sheared chromatin from 100,000 K562 cells using 1 µg the Diagenode antibody against H2A.Zac (Cat. No. C15410202) with the “iDeal ChIP-seq” kit (Cat. No. AB-001-0024) 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 1.5 Mb region of the X-chromosome (figure 2A and B) and in two regions surrounding the EIF4A2 and CCT5 positive control genes, respectively (figure 2C and D).

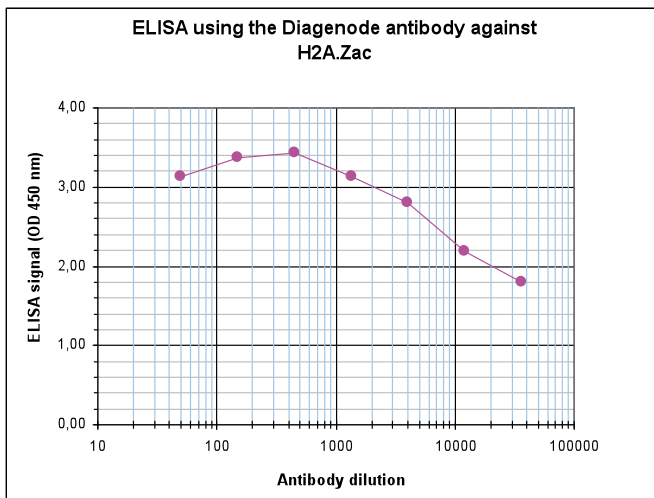


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against H2A.Zac (Cat. No. C15410202). 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 antibody was estimated to be 1:56,600.

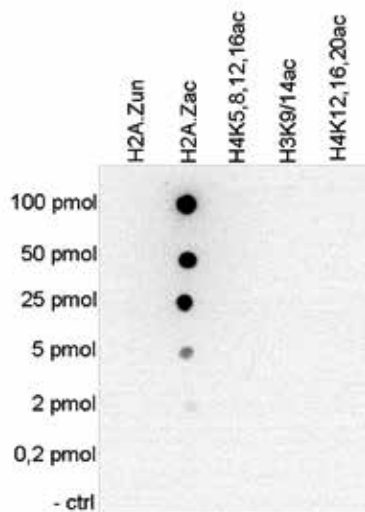


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H2A.Zac

To test the cross reactivity of the Diagenode antibody against H2A.Zac (Cat. No. C15410202), a Dot Blot analysis was performed with peptides containing different multiple acetylations and the unmodified H2A.Z. 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.

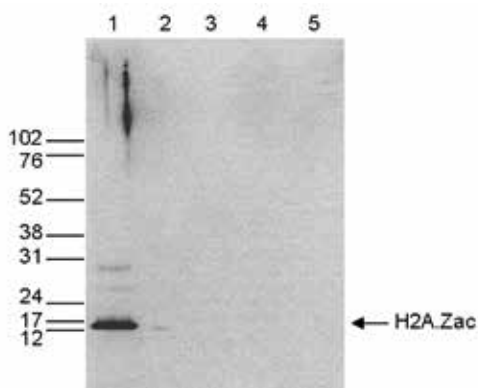


Figure 5. Western blot analysis using the Diagenode antibody directed against H2A.Zac

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. C15410202). 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. C15410202) 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 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.