

H2A.Z antibody

Cat. No. C15410201

Type: Polyclonal ChIP grade/ChIP-seq grade	Specificity: Human: positive Other species: not tested
Source: Rabbit	Purity: Affinity purified polyclonal antibody.
Lot: A2039P	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: 1.55 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 histone variant H2A.Z, using a KLH-conjugated synthetic peptide containing a sequence from the C-terminal part of the protein.

Applications

Applications	Suggested dilution	References
ChIP*	0.5 - 1 µg per IP	Fig 1, 2
CUT&TAG	1 µg	Fig 3
ELISA	1:5,000	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. Histone variant H2A.Z is associated with the active genes.

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Results

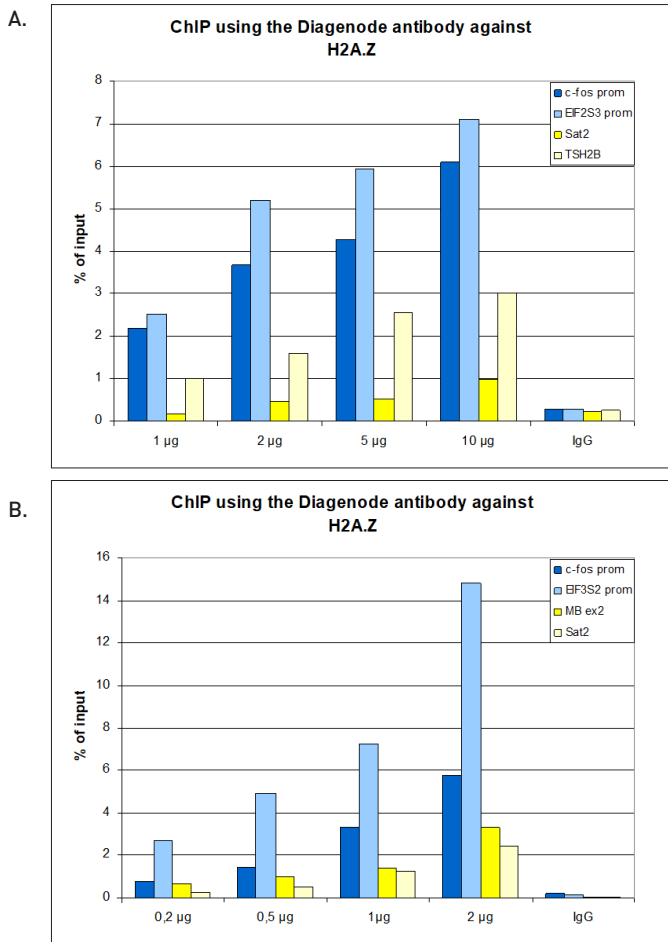
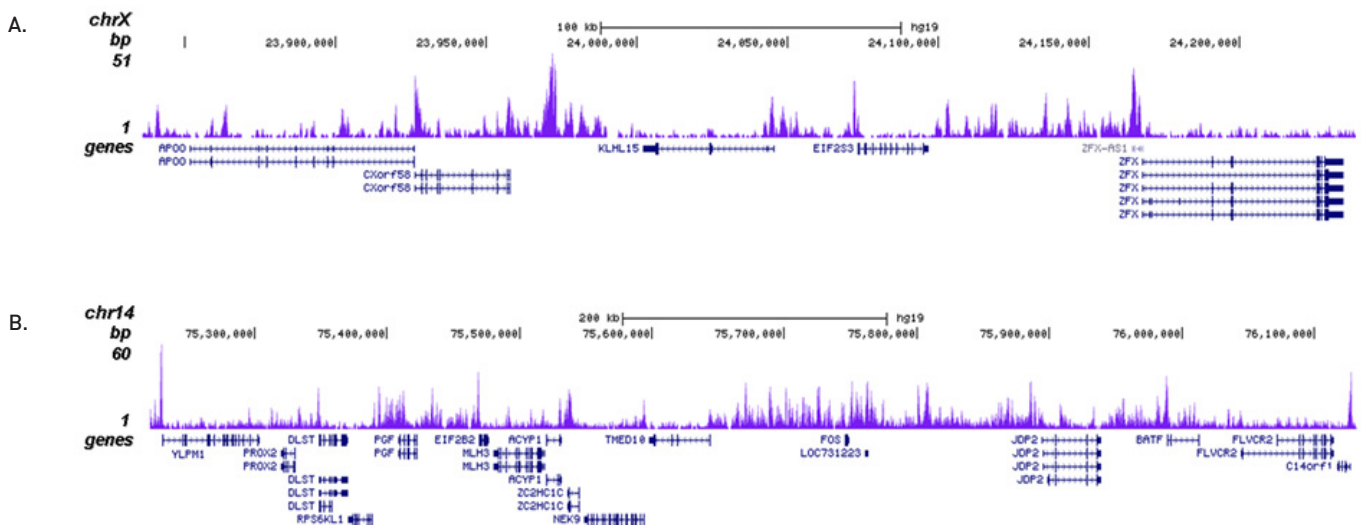


Figure 1. ChIP results obtained with the Diagenode antibody directed against H2A.Z

Figure 1A. ChIP assays were performed using human HeLa cells, the Diagenode antibody against H2A.Z (cat. No. C15410201) 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 specific for the promoter of the active genes *c-fos* and *EIF2S3*, used as positive controls, and for the inactive *TSH2B* gene and the *Sat2* satellite repeat, used as negative controls.

Figure 1B. ChIP assays were performed using human K562 cells, the Diagenode antibody against H2A.Z (cat. No. C15410201) and optimized PCR primer sets for qPCR. ChIP was performed with the “iDeal ChIP-seq” kit (cat. No. C01010051) 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 *c-fos* and *EIF2S3*, used as positive controls, and for the coding region of the inactive *MB* 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).



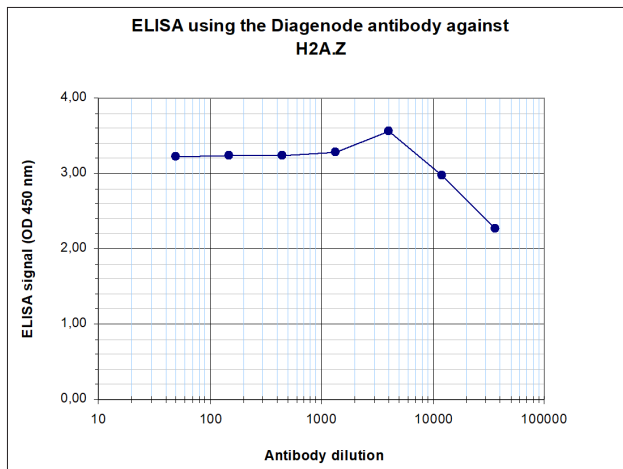


Figure 4. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against H2A.Z (cat. No. C15410201). 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 1:87,500.

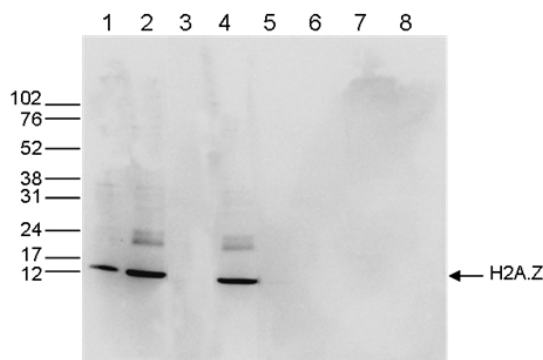


Figure 5. Western blot analysis using the Diagenode antibody directed against H2A.Z

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 5, 6, 7 and 8, respectively) using the Diagenode antibody against H2A.Z (cat. No. C15410201). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. Alternatively, Western blot was performed on histone extracts after incubation of the antibody with 1 µg of the peptide used for immunisation of the rabbit (1 hour at room temperature) (lane 3) or with a peptide containing a sequence from the central part of the H2A.Z protein (lane 4). The position of the protein of interest is indicated on the right, the marker (in kDa) is shown on the left.

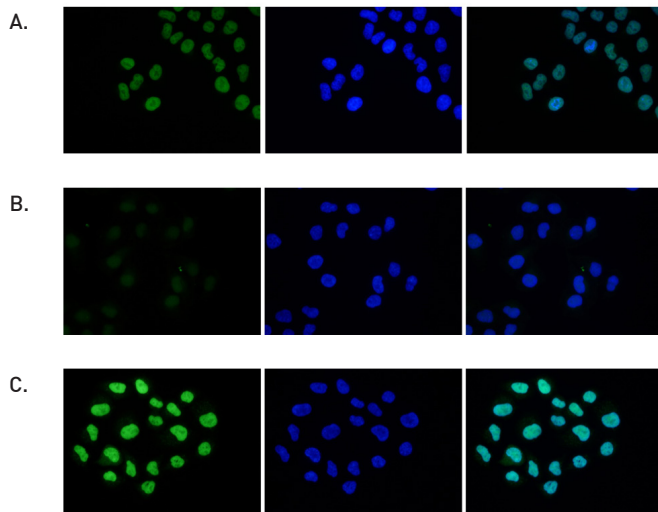


Figure 6. Immunofluorescence using the Diagenode antibody directed against H2A.Z

HeLa cells were stained with the Diagenode antibody against H2A.Z (cat. No. C15410201) 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. Figure 6A: cells were immunofluorescently labeled with the H2A.Z 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. Figure 6B and C: staining of the cells with the H2A.Z antibody after incubation of the antibody with 10 ng/µl of the peptide used for immunisation of the rabbit (figure 6B) and with a peptide containing a sequence from the central part of the H2A.Z protein (figure 6C).