diagenode
Innovating Epigenetic Solutions

ChIP-seq grade

TECHNICAL DATASHEET

H3K9/14ac polyclonal antibody

Cat. No. C15410005
Type: Polyclonal **ChIP grade/ChIP-seq grade**
Source: Rabbit
Lot #: A381-004
Size: 50 µg/ 36 µl
Concentration: 1.39 µg/µl

Specificity: Human, mouse, zebrafish, Nematodes, A. Nidulans, Arabidopsis: positive
Other species: not tested
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 histone H3 acetylated at lysines 9 and 14 (H3K9/14ac), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	**Results**
ChIP*	1 - 2 µg per ChIP	Fig 1, 2
ELISA	1:100	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:1,000	Fig 5
IF	1:500	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. Acetylation of H3K9/14 is enriched near the promoters of active genes.active genes.

Results

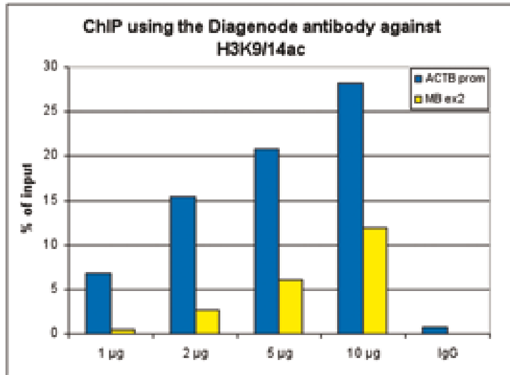


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K9/14ac

ChIP assays were performed using HeLa cells, the Diagenode antibody against H3K9/14ac (Cat. No. C15410005) and optimized primer pairs for qPCR. ChIP was performed with the "HighCell# ChIP" kit (Cat. No. C01010062), using sheared chromatin from 1.5 million cells. A titration of the antibody consisting of 1, 2, 5 and 10 µg per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control.QPCR was performed using primers specific for the promoter of the ACTB gene (Cat. No. C17011005) as a positive control target and for exon 2 of the MB gene (Cat. No. C17011006) as a negative control target. Figure 1 shows the recovery (the relative amount of immunoprecipitated DNA compared to input DNA). These results confirm the observation that acetylation of H3K9/14 is present at active promoters.

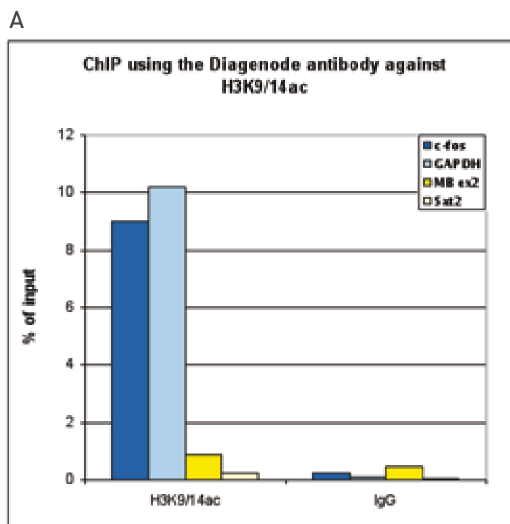
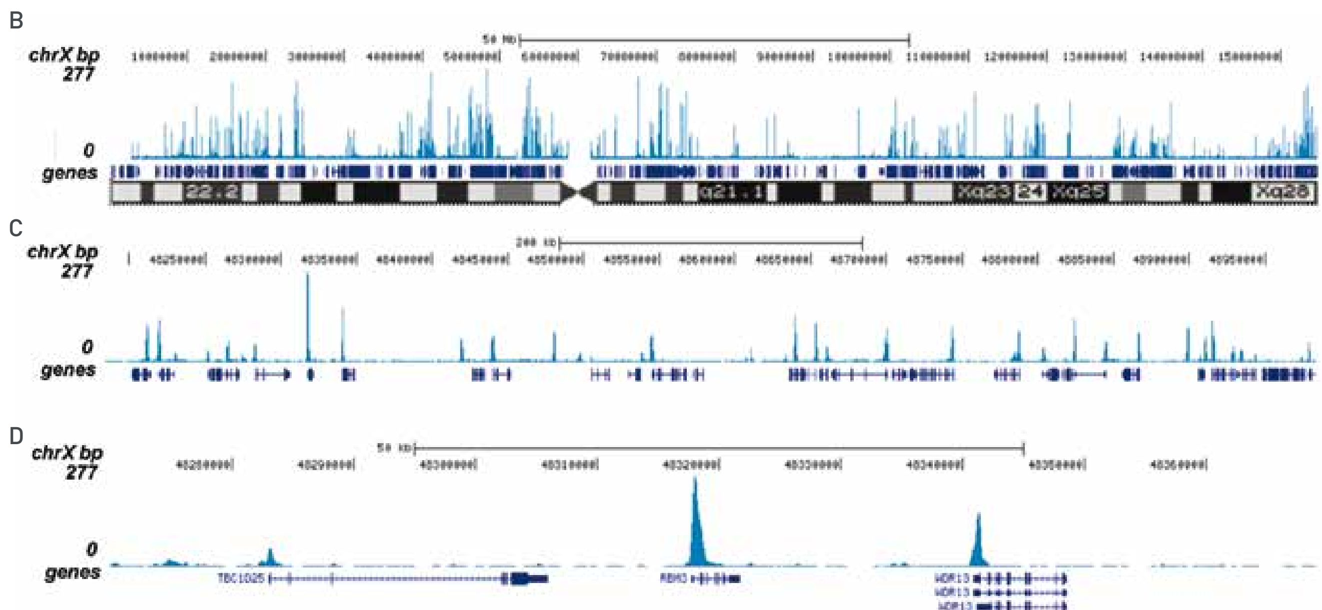
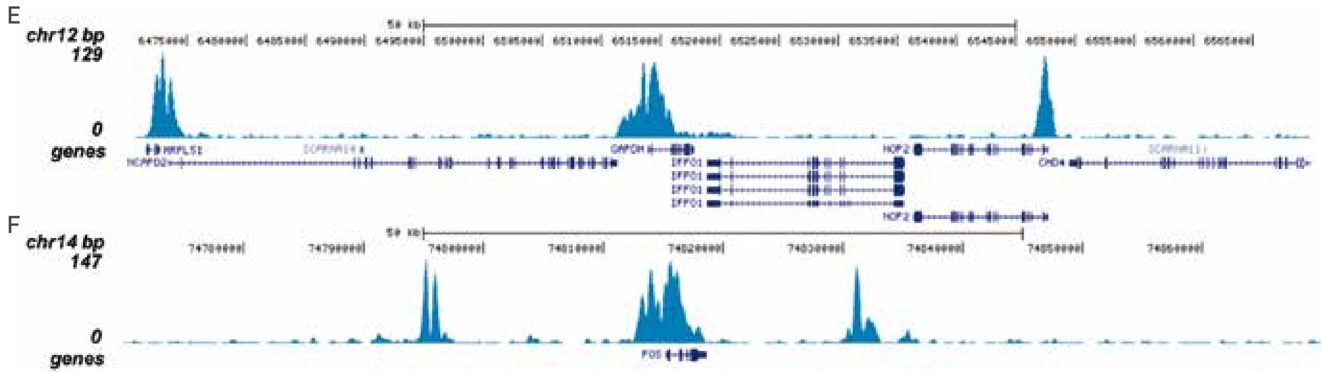


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K9/14ac

ChIP was performed with 1 µg of the Diagenode antibody against H3K9/14ac (Cat. No. C15410005) on sheared chromatin from 1 million HeLaS3 cells using the "Auto Histone ChIP-seq" kit (Cat. No. C01010022) on the IP-Star automated system. IgG (2 µg/IP) was used as a negative IP control. The IP'd DNA was analysed by QPCR with optimized PCR primer pairs for the promoters of the active GAPDH and c-fos genes, used as positive control targets, and the coding region of the inactive MB gene and the Sat2 satellite repeat, used as negative control targets (figure 2A). 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 800 kb region of the X-chromosome (figure 2B and C) and in 100 kb regions surrounding the RBM3, GAPDH and c-fos genes (figure 2D, E and F). These results clearly show an enrichment of the H3K9/14 double acetylation at the promoters of active genes.





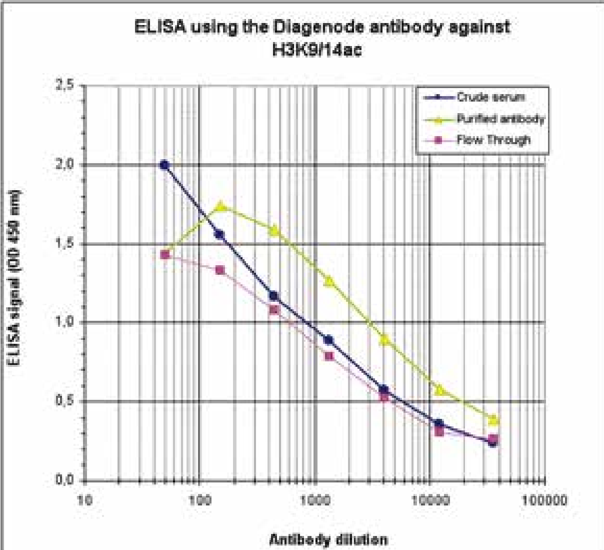


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K9/14ac (Cat. No. C15410005), crude serum and flow through 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 3), the titer of the purified antibody was estimated to be 1:5,900.

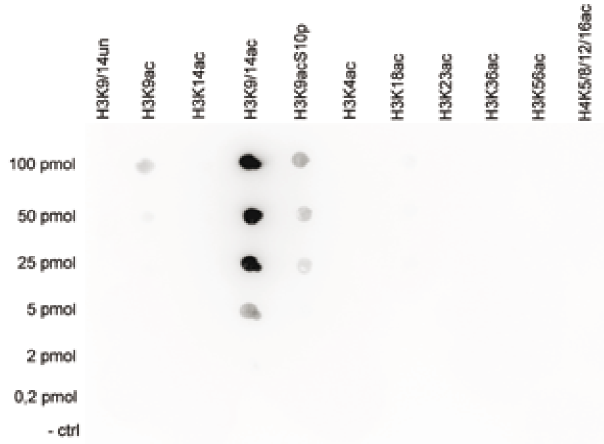


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K9/14ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9/14ac (Cat. No. C15410005) with peptides containing other histone modifications and the unmodified H3K9/14 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

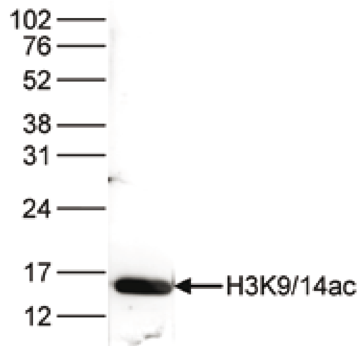


Figure 5. Western blot analysis using the Diagenode antibody directed against H3K9/14ac

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody directed against H3K9/14ac (Cat. No. C15410005) 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.

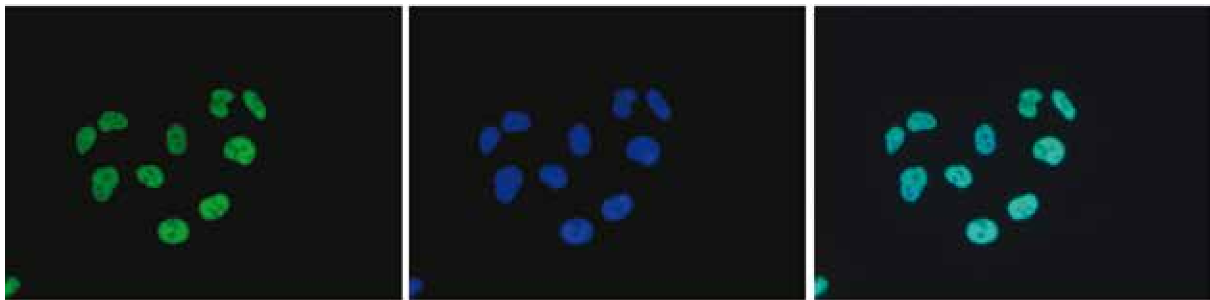


Figure 6. Immunofluorescence using the Diagenode antibody directed against H3K9/14ac

Mouse NIH3T3 cells were stained with the Diagenode antibody against H3K9/14ac (Cat. No. C15410005) 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 H3K9/14ac 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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