diagenode
Innovating Epigenetic Solutions

H4K8ac polyclonal antibody

Cat. No. C15410103 (pAb-103-050)
Type: Polyclonal ChIP-grade
Source: Rabbit
Lot #: A157-004
Size: 50 µg/ 41 µl
Concentration: 1.23 µg/µl

Specificity: Human, mouse: 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 the region of histone H4 containing the acetylated lysine 8 (H4K8ac), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	References
ChIP*	1 µg/ChIP	Fig 1
ELISA	1:100	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:200	Fig 4
IF	1:500	Fig 5

* 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.

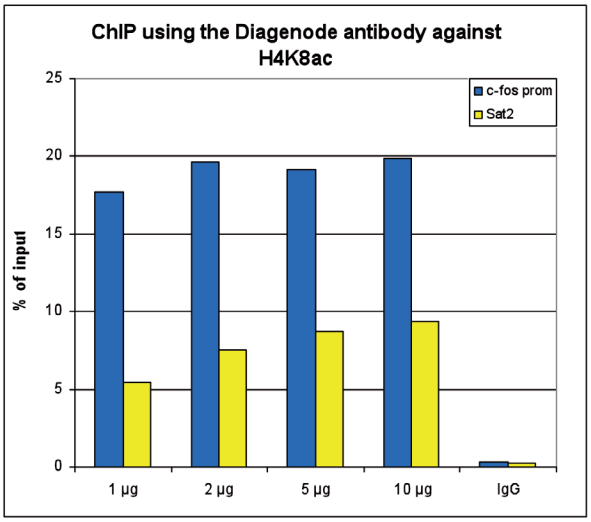

Figure 1. ChIP results obtained with the Diagenode antibody directed against H4K8ac

ChIP assays were performed using human HeLa cells, the Diagenode antibody directed against H4K8ac (cat. No. pAb-103-050) and optimized PCR primer sets for qPCR. ChIP was performed with the "LowCell# ChIP" kit (cat. No. kch-maglow-016) on sheared chromatin from 10,000 cells using the SX-8G IP-Star automated system. A titration of the antibody consisting of 1, 2, 5, and 10 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the c-fos promoter and for the Sat2 satellite repeat region. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

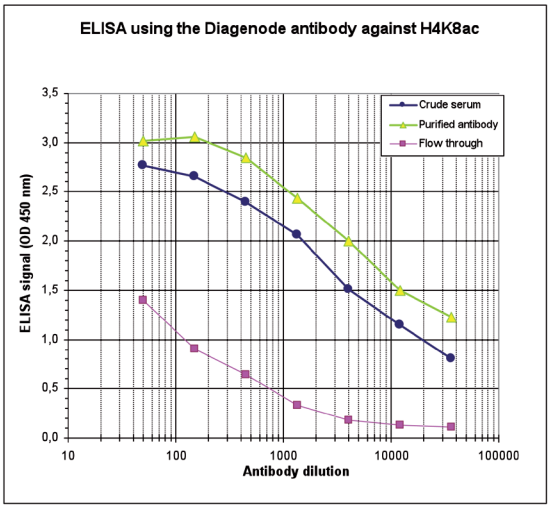

Figure 2. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H4K8ac (cat. No. pAb-103-050), 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 2), the titer of the purified antibody was estimated to be 1:16,700.

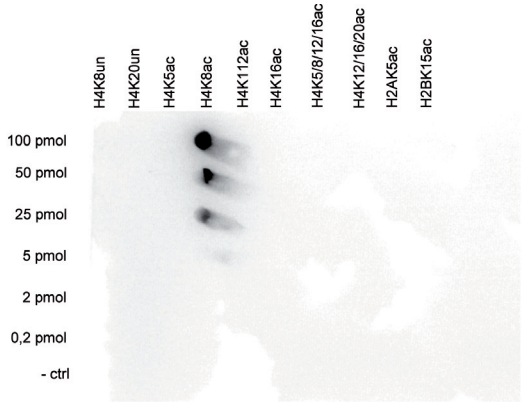

Figure 3. Cross reactivity test using the Diagenode antibody directed against H4K8ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H4K8ac (cat. No. pAb-103-050) with peptides containing other histone modifications and the unmodified H4K8. 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 3 shows a high specificity of the antibody for the modification of interest.

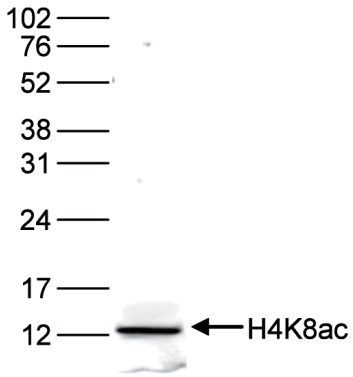

Figure 4. Western blot analysis using the Diagenode antibody directed against H4K8ac

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody directed against H4K8ac (cat. No. pAb-103-050) 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.

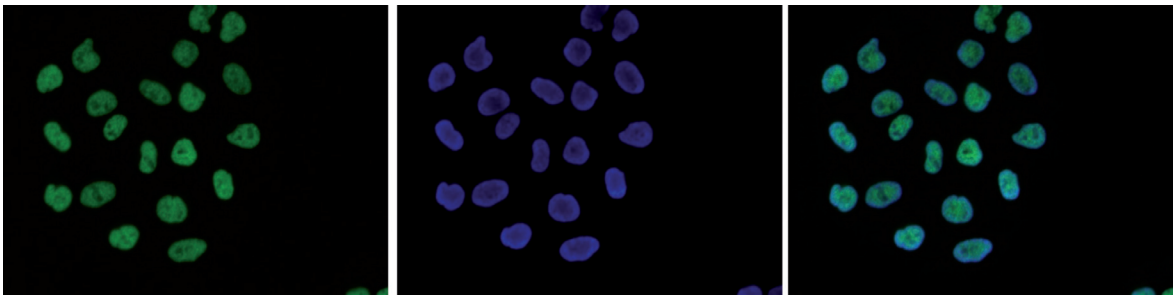

Figure 5. Immunofluorescence using the Diagenode antibody directed against H4K8ac

Mouse NIH3T3 cells were stained with the Diagenode antibody against H4K8ac (cat. No. pAb-103-050) 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 H4K8ac 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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