diagenode
Innovating Epigenetic Solutions

H3K4ac polyclonal antibody

Cat. No. C15410165 (pAb-165-050)
Type: Polyclonal **ChIP-grade**
Source: Rabbit
Lot #: A482-0042
Size: 50 µg/ 32 µl
Concentration: 1.58 µg/µl

Specificity: Human, Yeast: 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 lysine 4 (H3K4ac), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP*	0.5 µg/ChIP	Fig 1
ELISA	1:500	Fig 2
Dot blotting	1:10,000	Fig 3
Western blotting	1:500	Fig 4
Immunofluorescence	1:300	Fig 5

* Please note that the optimal antibody amount per IP should be determined by the end-user.

References

This antibody has been described in:

(1) Xhemalce B and Kouzarides T (2010) A chromodomain switch mediated by histone H3 Lys 4 acetylation regulates heterochromatin assembly. Genes Dev 24: 647-652.

Product 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.

Results

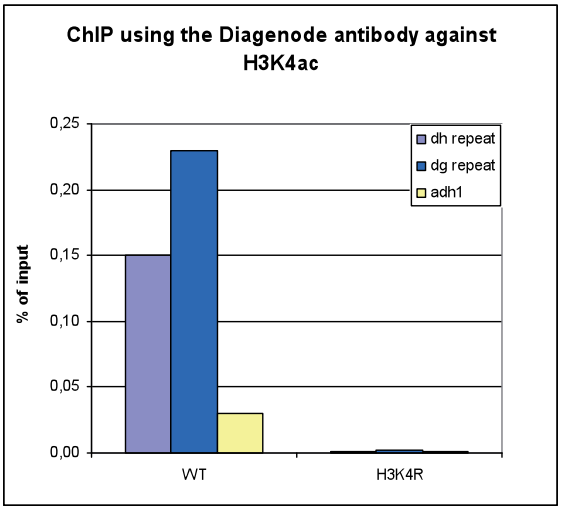

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4ac

ChIP assays were performed using WT and H3K4R mutant S. pombe cells, the Diagenode antibody against H3K4ac (Cat. No. C15410165) and optimized primer pairs for qPCR. Sheared chromatin corresponding to 10 µg of DNA and 0.5 µg of antibody were used per ChIP experiment. QPCR was performed using primers specific for two different pericentric repeat regions and for the euchromatic adh1 gene. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA).

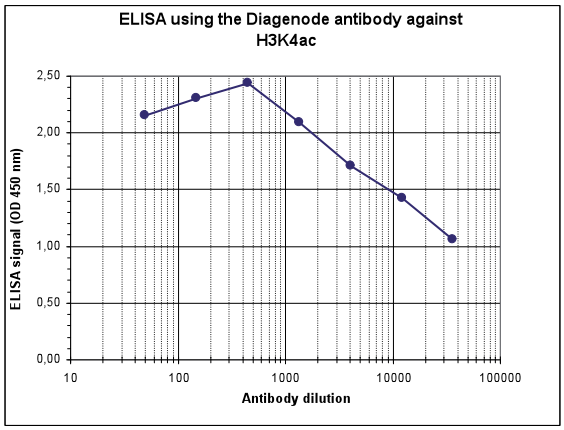

Figure 2. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K4ac (Cat. No. C15410165) 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 1:27,800.

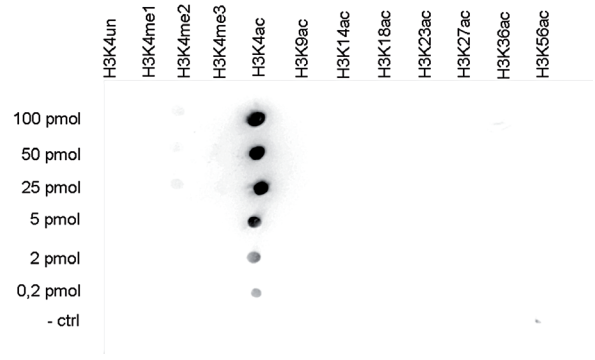

Figure 3. Cross reactivity test using the Diagenode antibody directed against H3K4ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K4ac (Cat. No. C15410165) with peptides containing other histone H3 modifications and the unmodified H3K4 sequence. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:10,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

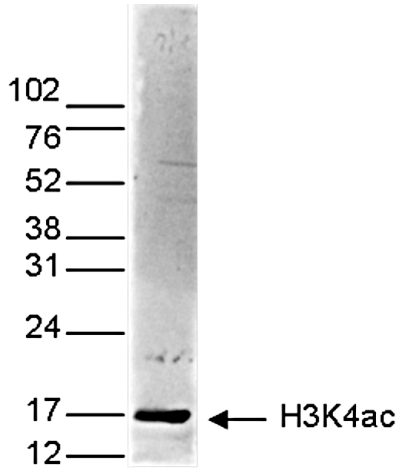

Figure 4. Western blot analysis using the Diagenode antibody directed against H3K4ac

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode antibody directed against H3K4ac (Cat. No. C15410165), diluted 1:500 in TBS-Tween containing 5% BSA. The marker (in kDa) is shown on the left, the position of the protein of interest is indicated on the right.

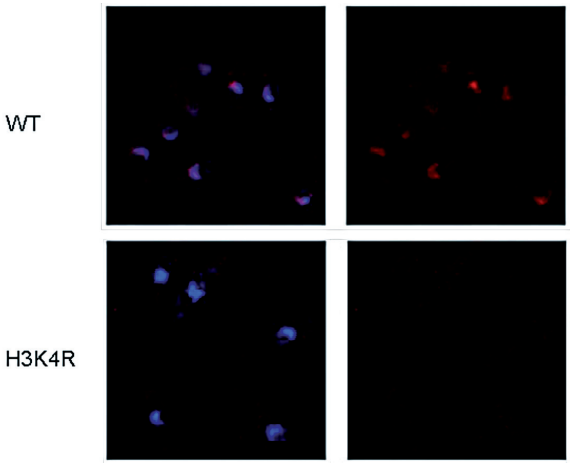

Figure 5. Immunofluorescence with the Diagenode antibody directed against H3K4ac

Wild type and H3K4R mutant S. pombe cells were stained with both the Diagenode antibody against H3K4ac (Cat. No. C15410165) (in red) and by Hoechst staining (in blue, left), or with the H3K4ac antibody alone (right). The antibody was used at a dilution of 1:300.

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