

PRODUCT NAME H3K9/14ac polyclonal antibody		
Catalog #: pAb-005-044 (also pAb-ACHBHS-044)	Type: Polyclonal ChIP-grade	Size: 50 µg/ 36 µl
Lot #: A381-004	Source: Rabbit	Concentration: 1.39 µg/µl

Description: Polyclonal antibody raised in rabbit against histone H3 acetylated at lysines 9 and 14 (H3K9/14ac), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ELISA	1:100	Fig 1
Dot blotting	1:20,000	Fig 2
Western blotting	1:1,000	Fig 3
ChIP	2 µg per ChIP	Fig 4

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.

References citing this antibody:

- (1) Martens JHA, Brinkman AB, Simmer F, Francoijs K-J, Nebbioso A, Ferrara F, Altucci L, and Stunnenberg HG [2010] PML-RARα/RXR Alters the Epigenetic Landscape in Acute Promyelocytic Leukemia. *Cancer Cell* 17, 173-185.
- (2) Vincent A, Perrais M, Desseyn JL, Aubert JP, Pigny P and Van Seuningen I [2007] Epigenetic regulation (DNA methylation, histone modifications) of the 11p15 mucin genes (MUC2, MUC5AC, MUC5B, MUC6) in epithelial cancer cells. *Oncogene* 26: 6566-6576.

Last data sheet update: May 6, 2010

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.

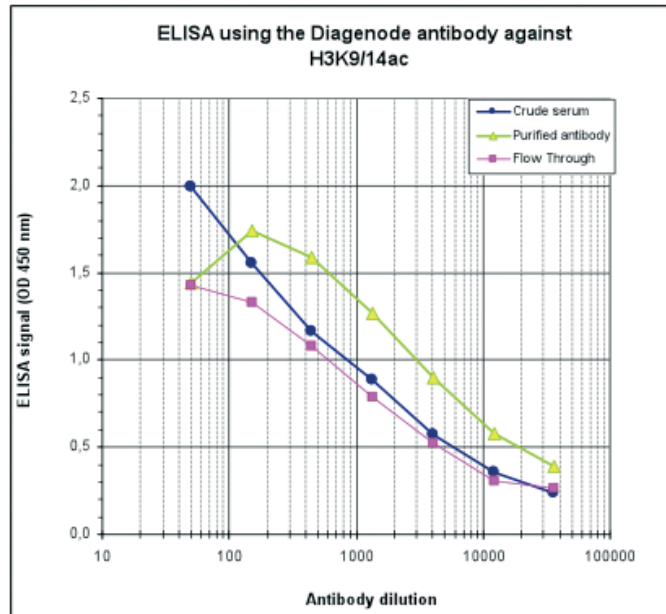


Figure 1
Determination of the antibody titer

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. pAb-005-044), 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 1), the titer of the purified antibody was estimated to be 1:5,900.

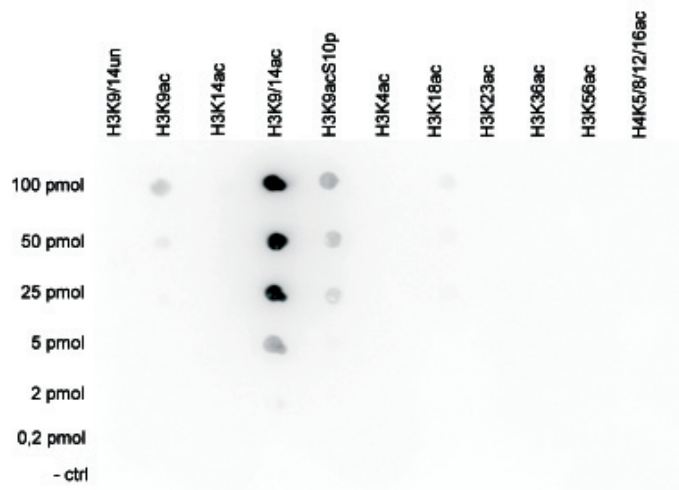


Figure 2
Cross reactivity test using the Diagenode antibody directed against H3K9/14ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9/14ac (cat. No. pAb-005-044) 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 2 shows a high specificity of the antibody for the modification of interest

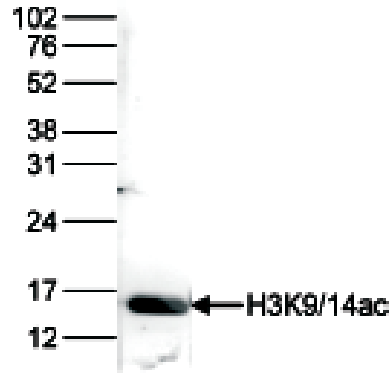


Figure 3

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. pAb-005-044) diluted 1:1,000 in TBS-Tween containing 5% skimmed milk (lane 1). The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

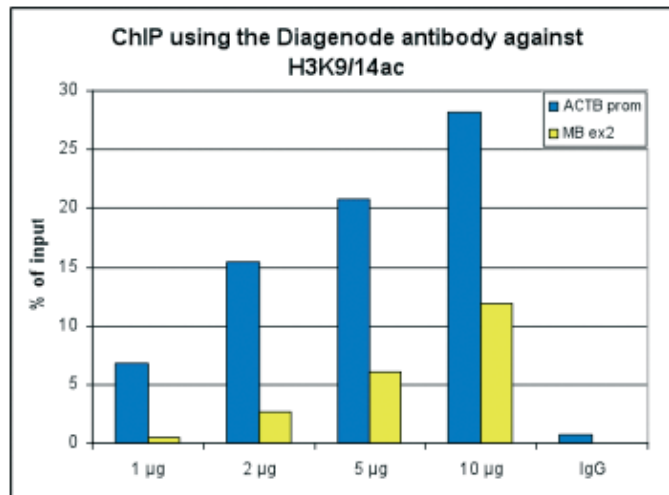


Figure 4

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. pAb-005-044) and optimized primer pairs for qPCR. ChIP was performed with the “HighCell# ChIP” kit (cat. No. kch-mahigh-A16), 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. pp-1005-050) as a positive control target and for exon 2 of the MB gene (cat. No. pp-1006-050) as a negative control target. Figure 3 shows the recovery (the relative amount of immunoprecipitated DNA compared to input DNA) and the occupancy (ratio +/- control target). These results confirm the observation that acetylation of H3K9/14 is present at active promoters.