diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME H3K9ac polyclonal antibody		
Cat. No. C15410177 (pAb-177-050)	**Type:** Polyclonal **ChIP-grade** **ChIP-seq grade**	**Size:** 50 µg/ 47 µl
Lot #: A1434-0012P	**Source:** Rabbit	**Concentration:** 1.08 µg/µl

Product description: Polyclonal antibody raised in rabbit against histone H3, acetylated at lysine 9 (H3K9ac), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP/ChIP-seq*	1- 2 µg per ChIP	Fig 1, 2
ELISA	1:1,000	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:750	Fig 5

*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

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.

Last data sheet update: May 18, 2011

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.

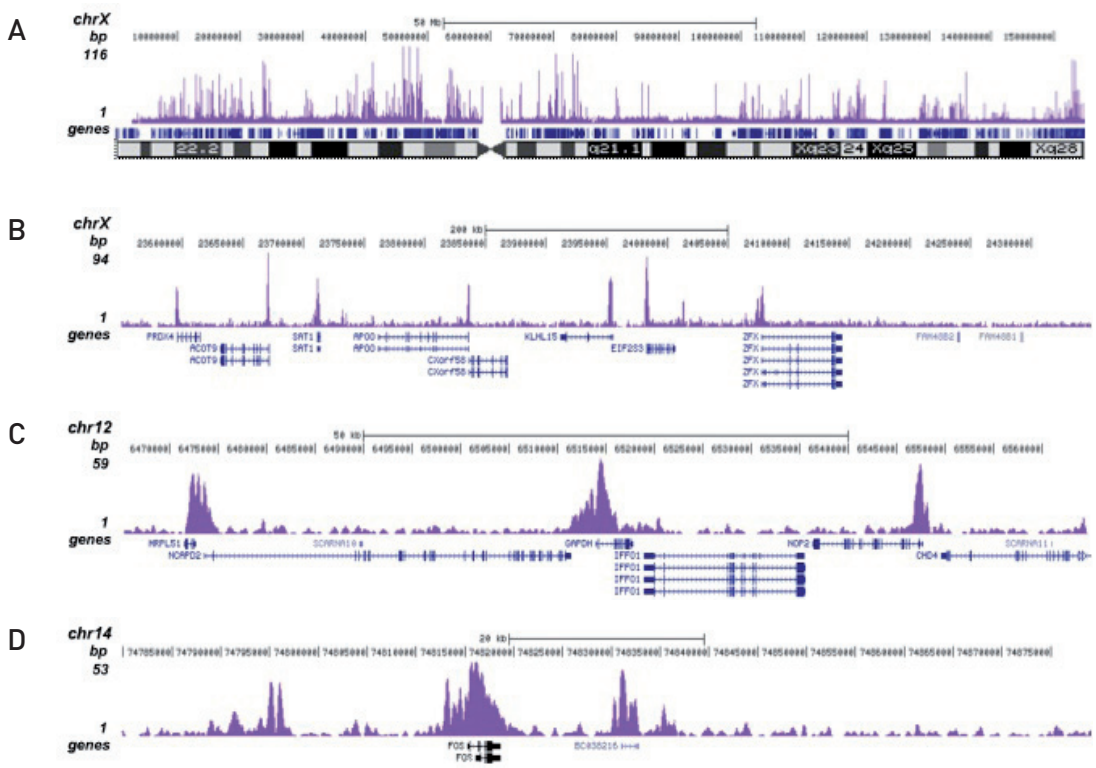

Figure 1

ChIP-seq results obtained with the Diagenode antibody directed against H3K9ac

ChIP was performed as described above using 1 µg of the Diagenode antibody against H3K9ac (cat. No. pAb-177-050). 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 2A and B) and in 100 kb regions surrounding the GAPDH and c-fos genes (figure 2C and D). These results clearly show an enrichment of H3K9ac at the promoters of active genes.

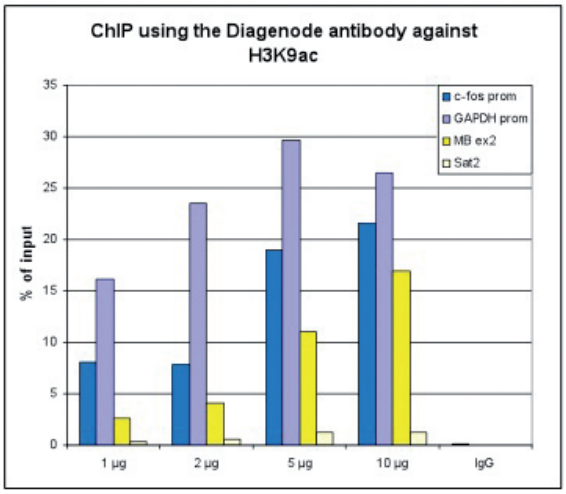

Figure 2

ChIP results obtained with the Diagenode antibody directed against RARA

ChIP assays were performed using HeLa cells, the Diagenode antibody against H3K9ac (cat. No. pAb-177-050) and optimized PCR primer sets for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit (cat. No. AB-Auto02-A100), using sheared chromatin from 1 million cells. A titration of the antibody consisting of 1, 2, 5 and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for the promoter of the active c-fos (cat. No. pp-1004-050) and GAPDH genes, used as positive controls, and for the coding region of the inactive MB gene (cat. No. pp-1006-050) 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). These results are in accordance with the observation that H3K9 acetylation is enriched at the promoters of active genes.

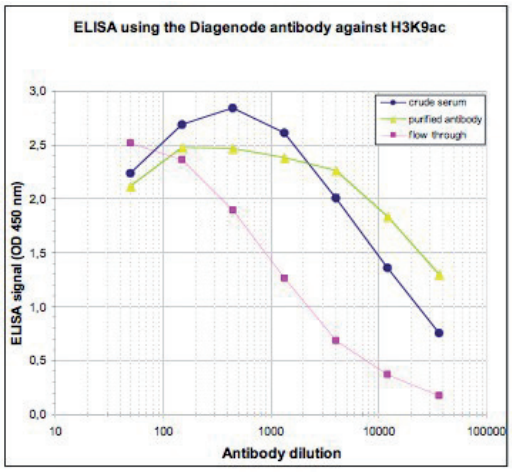

Figure 3

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 H3K9ac (cat. No. pAb-177-050) 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 antibody was estimated to be 1:59,600.

Figure 4

Cross reactivity test using the Diagenode antibody directed against H3K9ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9ac (cat. No. pAb-177-050) with peptides containing other histone modifications and the unmodified H3K9 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. Please note that this antibody recognizes the H3K9 acetylation, both in the presence and the absence of the K14 acetylation or the S10 phosphorylation.

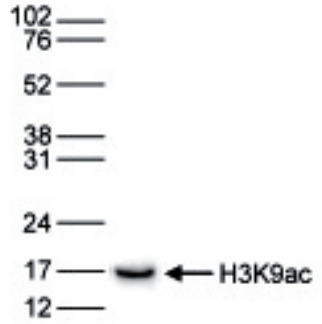

Figure 5

Western blot analysis using the Diagenode antibody directed against RARA

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K9ac (cat. No. pAb-177-050) 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.