TECHNICAL DATA SHEET

PRODUCT NAME H3K4me2 polyclonal antibody		
Cat. No. C15410035 (pab-035-050)	**Type:** Polyclonal **ChIP-grade / ChIP-seq grade**	**Size:** 50 µg/ 42 µl
Lot #: A936-0014P	**Source:** Rabbit	**Concentration:** 1.2 µg/µl

Product description: Polyclonal antibody raised in rabbit against histone H3 containing the dimethylated lysine 4 (H3K4me2), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP/ChIP-seq*	1 µg/ChIP	Fig 1, 2
ELISA	1:500	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:1,000	Fig 5
IF	1:5,000	Fig 6

*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 3, 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.

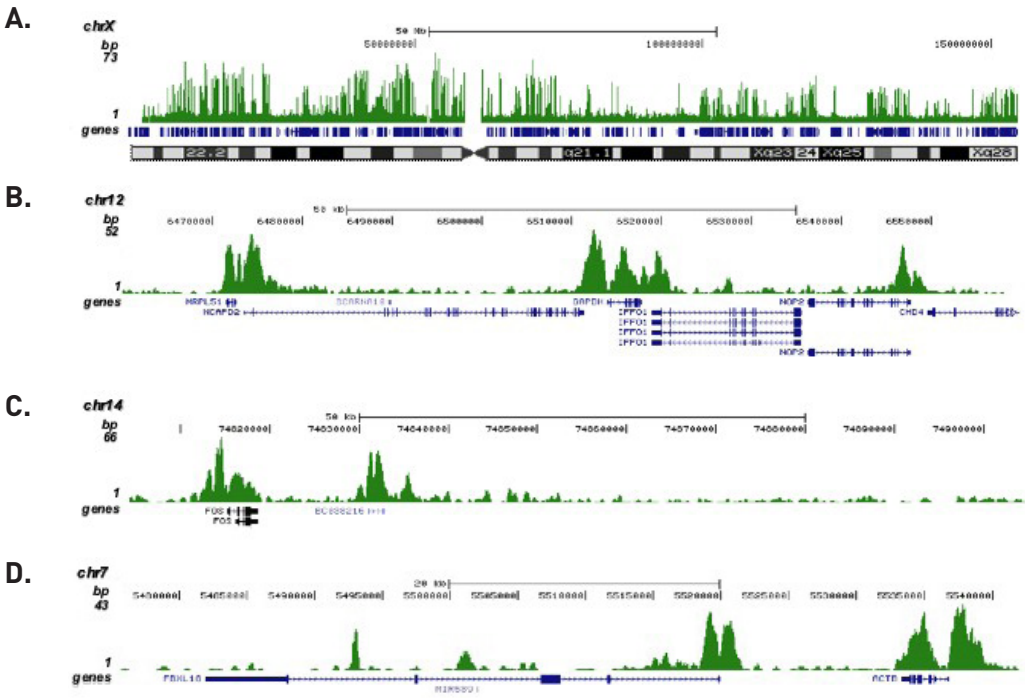

Figure 1

ChIP-seq results obtained with the Diagenode antibody directed against H3K4me2

ChIP was performed as described above using 1 µg of the Diagenode antibody against H3K4me2 (Cat. No. pAb-035-050). The IP'd DNA was analysed on 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 X-chromosome (figure 2A) and in 3 chromosomal regions surrounding the GAPDH, c-fos and ACTB genes (figure 2B, C and D, respectively).

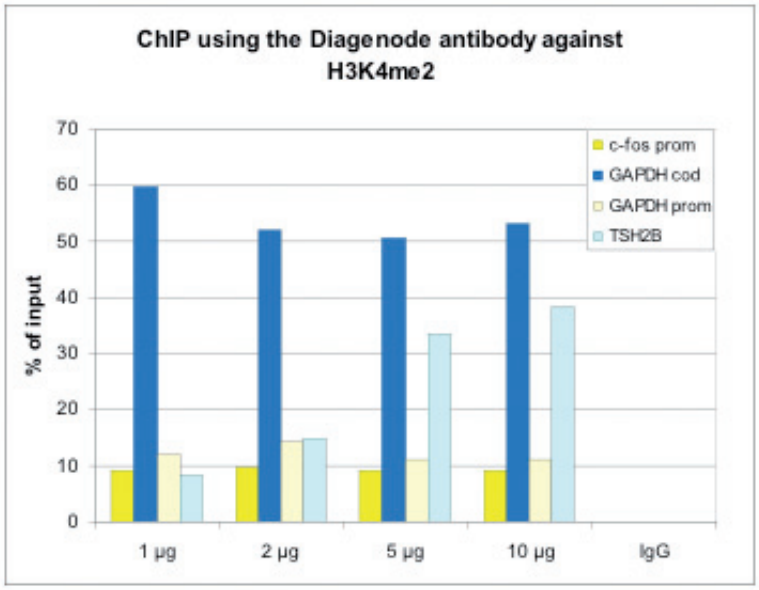

Figure 2

ChIP results obtained with the Diagenode antibody directed against H3K4me2

ChIP was performed with the Diagenode antibody against H3K4me2 (Cat. No. pAb-035-050) on sheared chromatin from 1 million HeLaS3 cells using the "Auto Histone ChIP-seq" kit. 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 and coding region of the active GAPDH gene, the promoter of the active c-fos gene and for the coding region of the inactive TSH2B. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

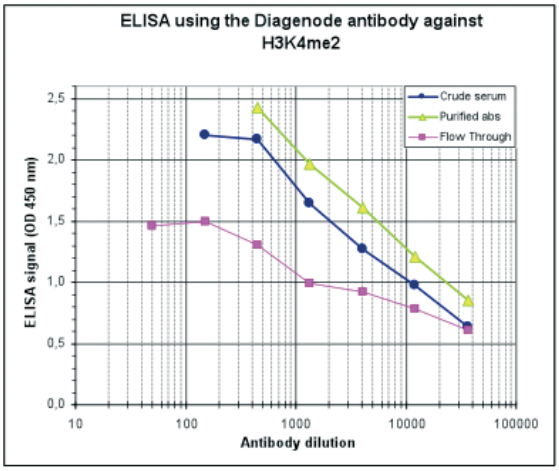

Figure 3
Determination of the titer
To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K4me2 (Cat. No. pAb-035-050), crude serum and flow through in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:12,600.

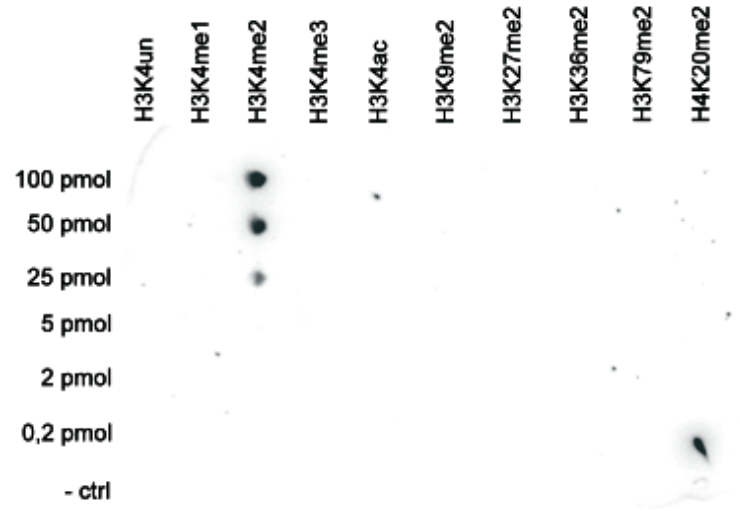

Figure 4
Cross reactivity test using the Diagenode antibody directed against H3K4me2
A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K4me2 (Cat. No. pAb-035-050) with peptides containing other modifications of histone H3 and H4 and the unmodified H3K4 sequence. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

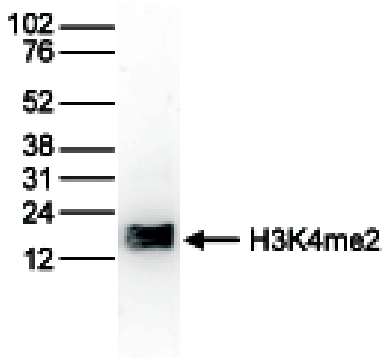

Figure 5

Western blot analysis using the Diagenode antibody directed against H3K4me2

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K4me2 (Cat. No. pAb-035-050) 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.