

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

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
ELISA	1:500	Fig 1
Dot blotting	1:20,000	Fig 2
Western blotting	1:1,000	Fig 3
ChIP	5 µg/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.

Last data sheet update: March 26, 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.

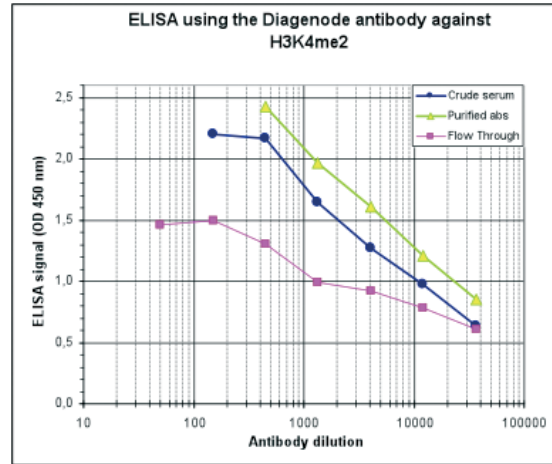


Figure 1
Determination of the titer

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 1), the titer of the antibody was estimated to be 1:12,600.

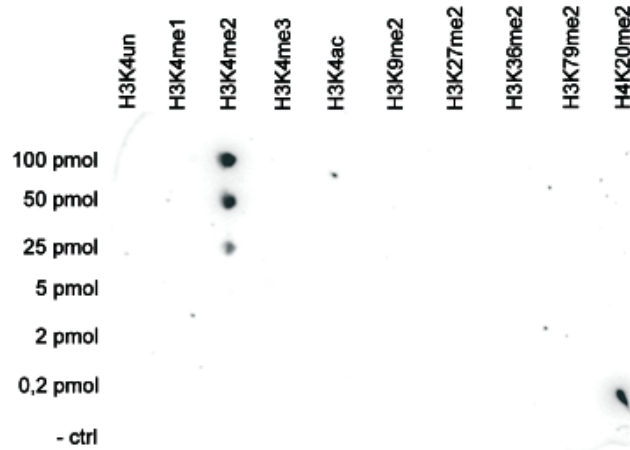


Figure 2
Cross reactivity test using the Diagenode antibody directed against H3K4me2

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 2 shows a high specificity of the antibody for the modification of interest.

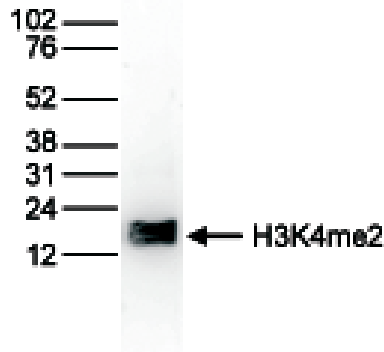


Figure 3

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 left; the marker (in kDa) is shown on the right.

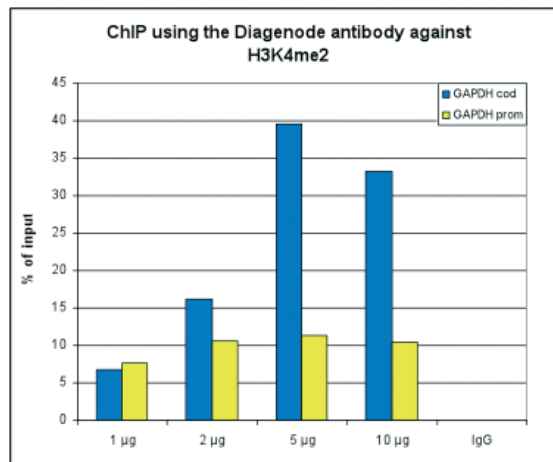


Figure 4

ChIP results obtained with the Diagenode antibody directed against H3K4me2

ChIP assays were performed using HeLaS3 cells, the Diagenode antibody against H3K4me2 [Cat. No. pAb-035-050] and optimized PCR primer sets for qPCR. ChIP was performed with the "OneDay ChIP" kit [Cat. No. kch-oneDIP-060], 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 (1 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for the promoter and for the coding region of GAPDH. Figure 4 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA).