diagenode
Innovating Epigenetic Solutions

H3K4me3 polyclonal antibody

Cat. No. C15310003
Type: Polyclonal **ChIP-grade**
Source: Rabbit
Lot #: A.49-001
Size: 100 µl
Concentration: Not determined

Specificity: Human, C. elegans: positive
Other species: not tested
Purity: Whole antiserum from rabbit containing 0.05% azide.
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 the region of histone H3 containing the trimethylated lysine 4 (H3K4me3), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP *	1 µl per ChIP	Fig 1
ELISA	1:100 - 1:500	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:1,000	Fig 4

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µl per IP.

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. Methylation of histone H3K4 is associated with activation of gene transcription.

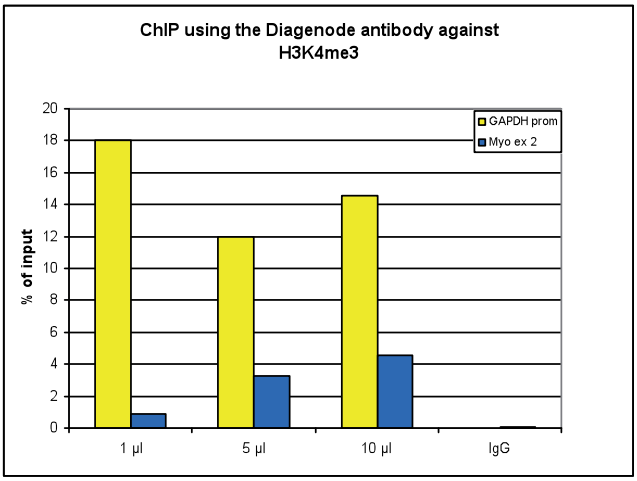

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4me3

ChIP assays were performed using human U2OS cells, the Diagenode antibody against H3K4me3 (Cat. No. C15310003) and optimized PCR primer pairs for qPCR. ChIP was performed with the "OneDay ChIP" kit (Cat. No. C01010080), using sheared chromatin from 2 million cells and stringent washing conditions. A titration consisting of 1, 5 and 10 µl of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers for the promoter of the constitutively expressed GAPDH gene and for myoglobin exon 2. 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 trimethylation of K4 at histone H3 is associated with the promoters of active genes.

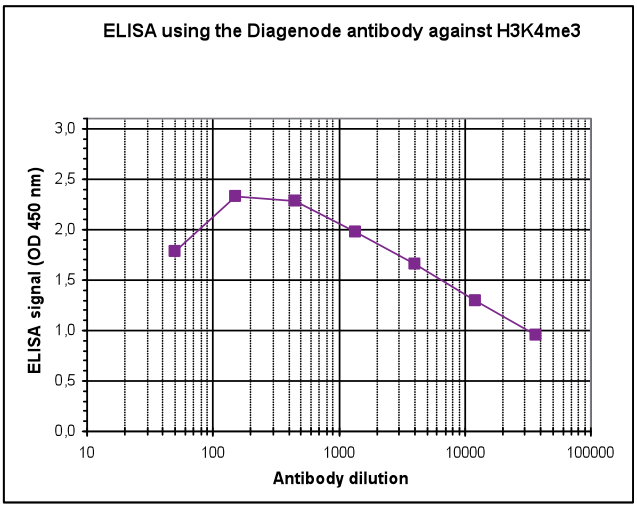

Figure 2. Determination of the antibody titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against human H3K4me3 (Cat. No. C15310003) 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 antibody was estimated to be 1:19,000.

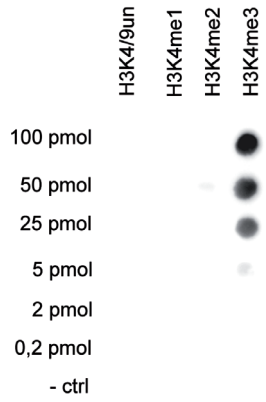

Figure 3. Cross reactivity tests using the Diagenode antibody directed against H3K4me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K4me3 (Cat. No. C15310003) with peptides containing other H3K4 methylations and the unmodified 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 3 shows a high specificity of the antibody for the modification of interest.

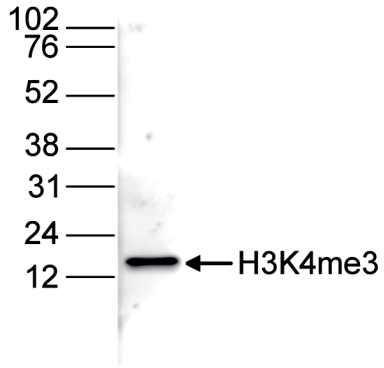

Figure 3. Western blot analysis using the Diagenode antibody directed against H3K4me3

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K4me3 (Cat. No. C15310003) 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.

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