

PRODUCT NAME H3K4me3 polyclonal antibody		
Cat. No. pAb-003-050	Type: Polyclonal ChIP-grade ChIP-seq-grade	Size: 50 µg/ 46 µl
Lot #: A3152-002P	Source: Rabbit	Concentration: 1.1 µg/µl

Description: Polyclonal antibody raised in rabbit against the region of histone H3 containing the trimethylated lysine 4 (H3K4me3), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
Dot blotting	1:10,000	Fig 1
Western blotting	1:500	Fig 2
ChIP	2 µg per IP	Fig 3, 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] Buschbeck M, Uribealago I, Wibowo I, Rué P, Martin D, Gutierrez A, Morey L, Guigo R, Lopez-Schier H and Di Croce L (2009) The histone variant macroH2A is an epigenetic regulator of key developmental genes. *Nat Struct Mol Biol* 16: 1074-1079.
- [2] Marks H, Chow JC, Denissov S, François KJ, Brockdorff N, Heard E and Stunnenberg HG (2009) High-resolution analysis of epigenetic changes associated with X inactivation. *Genome Res* 19: 1361-1373.

Last data sheet update: November 08, 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. Methylation of histone H3K4 is associated with activation of gene transcription.

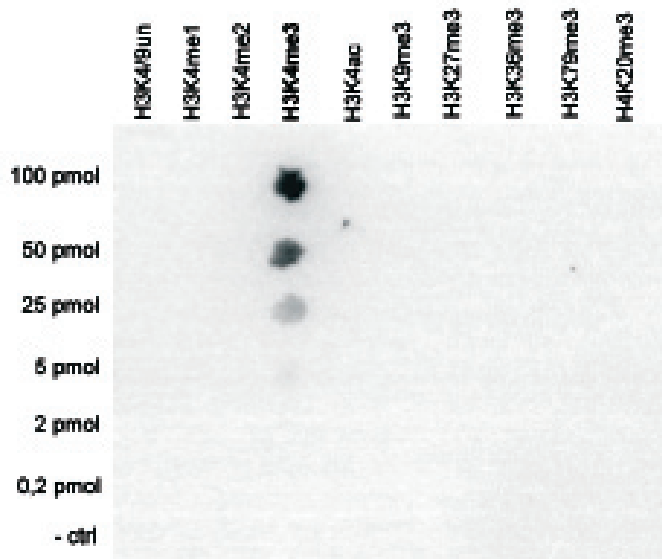


Figure 1

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. pAb-003-050] with peptides containing other histone modifications and the unmodified H3K4. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:10,000. Figure 1 shows a high specificity of the antibody for the modification of interest."

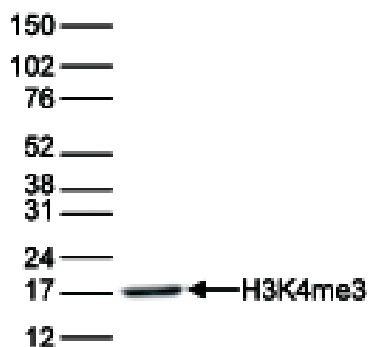


Figure 2

Western blot analysis using the Diagenode antibody against H3K4me3

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K4me3 [cat. No. pAb-003-050] diluted 1:500 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.

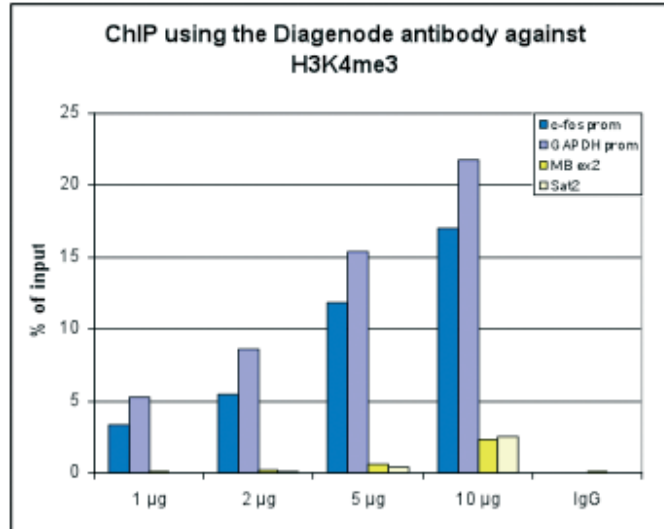


Figure 3

ChIP results obtained with the Diagenode antibody directed against H3K4me3

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K4me3 (cat. No. pAb-003-050) and optimized PCR primer pairs for qPCR. ChIP was performed with the “HighCell# ChiP” kit (cat. No. kch-mahigh-A16), using sheared chromatin from 2 million cells on the SX-8G IP-Star automated system. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (5 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoter of the constitutively expressed GAPDH and c-fos genes, used as positive controls, and for exon 2 of the inactive myoglobin (MB) gene and the Sat2 satellite repeat, used as negative controls. Figure 3 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.

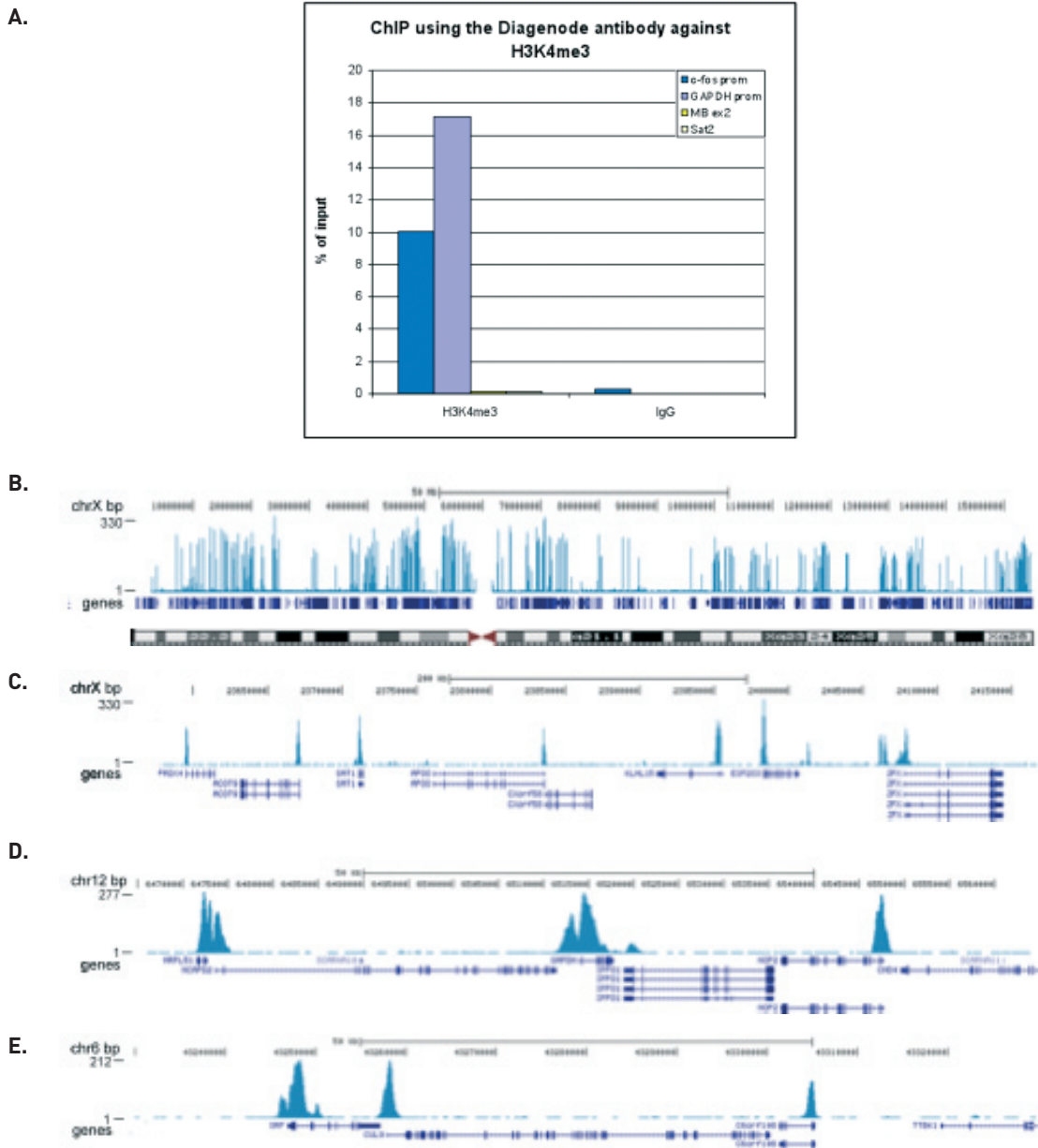


Figure 4

ChIP-seq results obtained with the Diagenode antibody directed against H3K4me3

ChIP was performed on sheared chromatin from 1 million HeLaS3 cells with the “Auto Histone ChIP-seq” kit using 2 µg of the Diagenode antibody against H3K4me3 (cat. No. pAb-003-050). IgG (2 µg/IP) was used as a negative IP control. The IP’d DNA from 6 separate reactions was pooled and analysed by QPCR as described (figure 4A). The IP’d DNA was subsequently 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 4 shows the peak distribution along the complete sequence and a 600 kb region of the X-chromosome (figure 4B and C) and in 100 kb regions surrounding the GAPDH and c-fos (SRF) positive control genes (figure 4D and E). These results clearly show an enrichment of the H3K4 trimethylation at the promoters of active genes.