diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K4me3 antibody

Cat. No. C15200152

Type: Monoclonal **ChIP-seq grade**
Isotype IgG1
Source: Mouse
Lot: 001-15
Size: 50 µg
Concentration: 1 µg/µl

Specificity: Human, Mouse, Nematodes, Arabidopsis: positive.
Other species: not tested.
Purity: Protein A purified monoclonal antibody.
Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Storage buffer: PBS containing 0.05% azide.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Monoclonal antibody raised in mouse against histone H3, trimethylated at lysine 4 (H3K4me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	2 µg per ChIP	Fig 1,2
CUT&TAG	1 µg	Fig 3
ELISA	1:3,000	Fig 4
Western blotting	1:1,000	Fig 5
Immunofluorescence	1:500	Fig 6

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

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Results

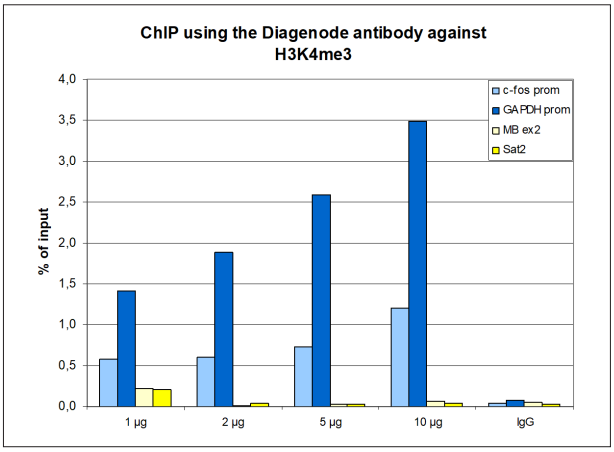

Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3K4me3

ChIP assays were performed using human HeLa cells, the Diagenode monoclonal antibody against H3K4me3 (cat. No. C15200152) and optimized PCR primer pairs for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit (cat. No. C01010022), using sheared chromatin from 1 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 (2 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 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.

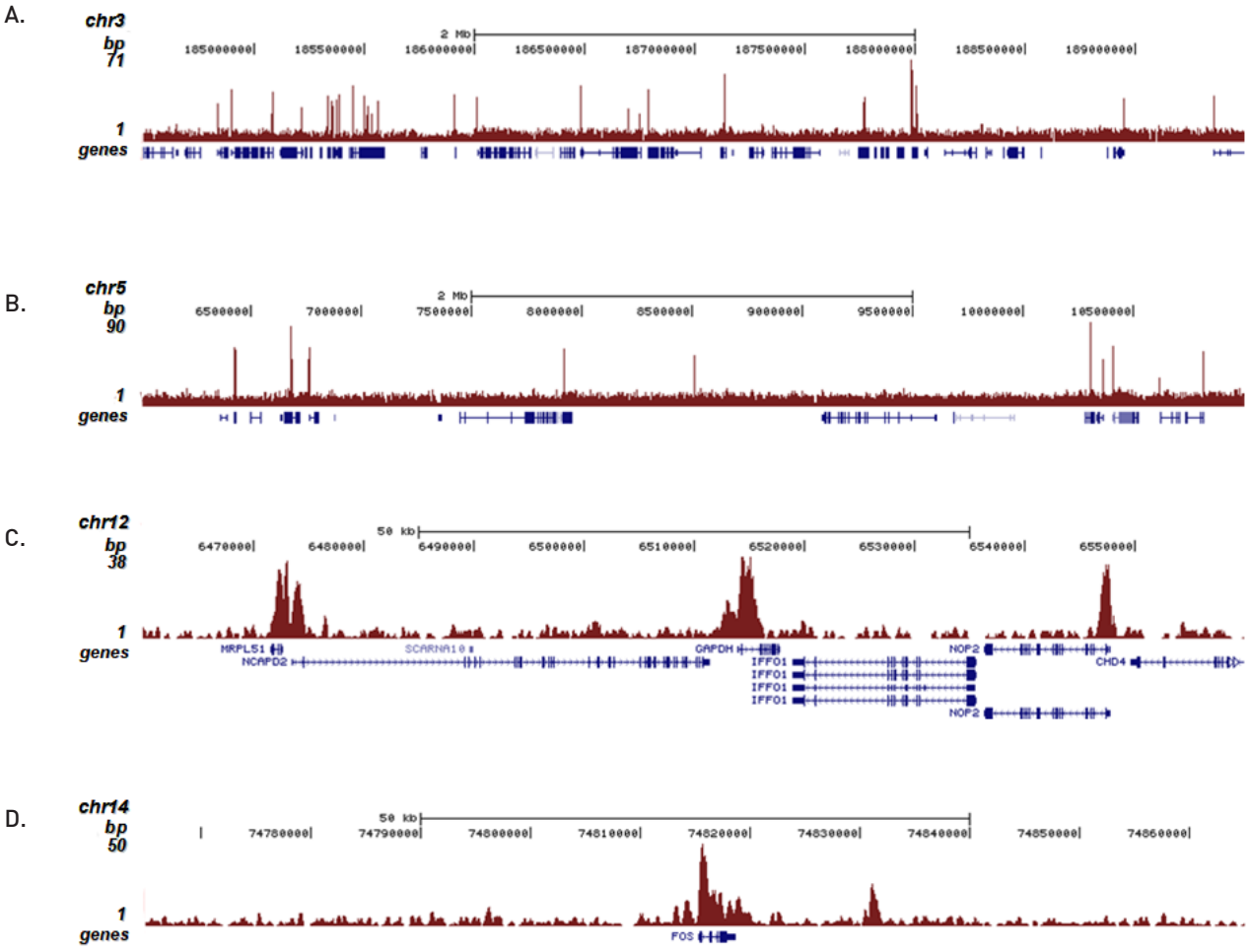

Figure 2. ChIP-seq results obtained with the Diagenode monoclonal antibody directed against H3K4me3

ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 2 µg of the Diagenode antibody against H3K4me3 (cat. No. C15200152) as described above. 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 2 shows the peak distribution along two 5 Mb regions of chromosome 3 and 5 (figure 2A and B, respectively) and in two 100 kb regions surrounding the GAPDH and c-fos positive control genes (figure 2C and D). These results clearly show an enrichment of the H3K4 trimethylation at the promoters of active genes.

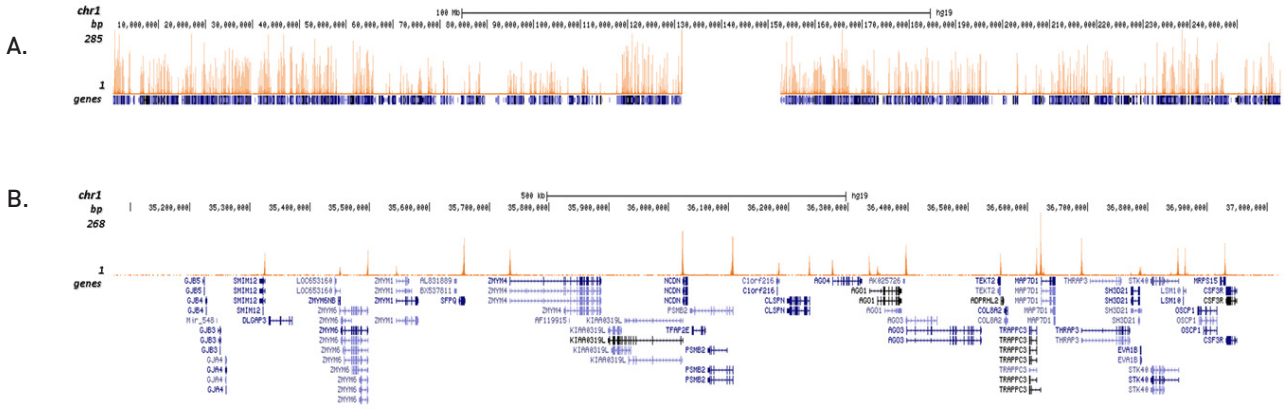

Figure 3. Cut&Tag results obtained with the Diagenode monoclonal antibody directed against H3K4me3

CUT&TAG (Kaya-Okur, H.S., Nat Commun 10, 1930, 2019) was performed on 50,000 K562 cells using 1 µg of the Diagenode monoclonal antibody against H3K4me3 (cat. No. C15200152) and the Diagenode pA-Tn5 transposase (C01070001). The libraries were subsequently analysed on an Illumina NextSeq 500 sequencer (2x75 paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg19) using the BWA algorithm. Figure 3 shows the peak distribution along the complete sequence and a 1.5 Mb zoomin of chromosome 1 (figure 3A and B, respectively).

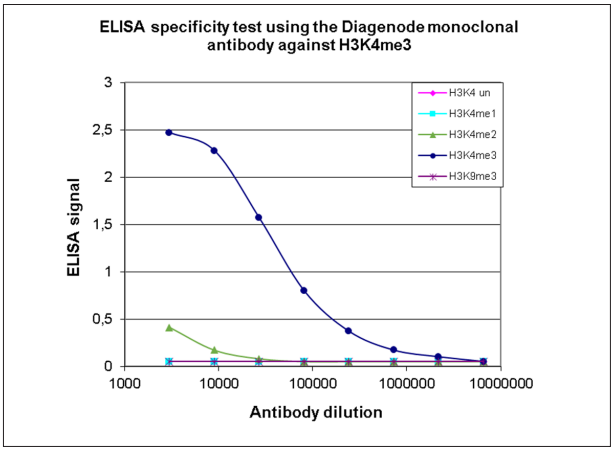

Figure 4. Cross reactivity of the Diagenode monoclonal antibody directed against H3K4me3

To test the specificity an ELISA was performed using a serial dilution of the Diagenode monoclonal antibody against H3K4me3 (cat. No. C15200152). The wells were coated with peptides containing the unmodified H3K4 as well as the mono-, di- and trimethylated H3K4 and the trimethylated H3K9. Figure 4 shows a high specificity of the antibody for the modification of interest.

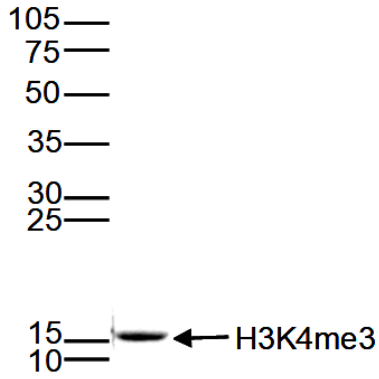

Figure 5. Western blot analysis using the Diagenode monoclonal antibody directed against H3K4me3

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode monoclonal antibody against H3K4me3 (cat. No. C15200152) 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.

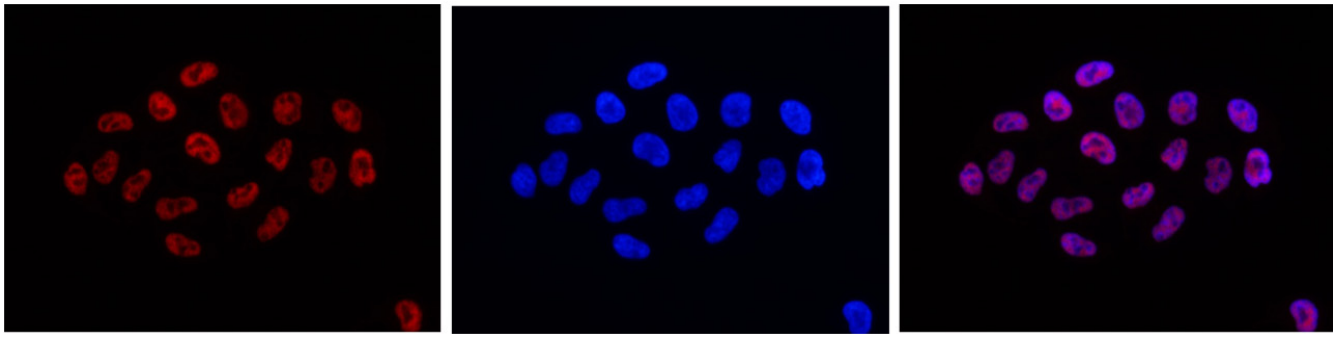

Figure 6. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K4me3

HeLa cells were stained with the Diagenode antibody against H3K4me3 (cat. No. C15200152) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K4me3 antibody (left) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.