diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K4me2 antibody

Cat. No. C15410035

Type: Polyclonal **ChIP grade/ChIP-seq grade**
Source: Rabbit
Lot: A936-0023
Size: 50 µg
Concentration: 1.1 µg/µl

Specificity: Human, Arabidopsis: positive
Other species: not tested
Purity: Affinity purified polyclonal 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: Polyclonal antibody raised in rabbit against histone H3 containing the dimethylated lysine 4 (H3K4me2), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	0.5 - 1 µg per IP	Fig 1, 2
CUT&TAG	0.5 µg	Fig 3
ELISA	1:100 - 1:500	Fig 4
Dot blotting	1:2,000	Fig 5
Western blotting	1:1,000	Fig 6

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5-5 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

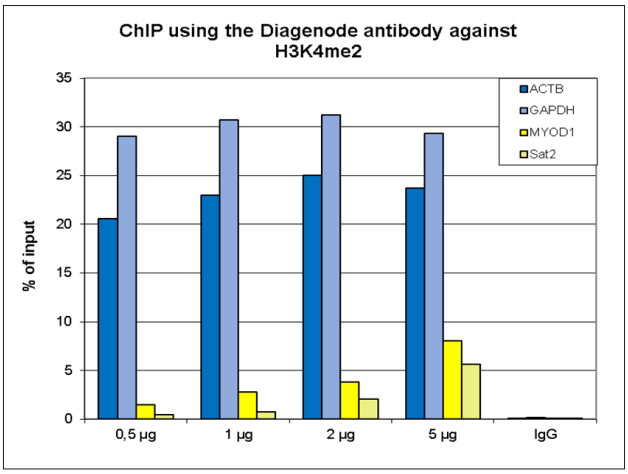

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4me2

ChIP was performed with the Diagenode antibody against H3K4me2 (cat. No. C15410035) on sheared chromatin from 500,000 K562 cells using the "iDeal ChIP-seq" kit (cat. No. C01010051). A titration of the antibody consisting of 0.5, 1, 2 and 5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for a region upstream of the ACTB and GAPDH promoters, used as positive controls, and for the MYOD1 gene and the Sat2 satellite repeat, used as negative controls. The graph shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

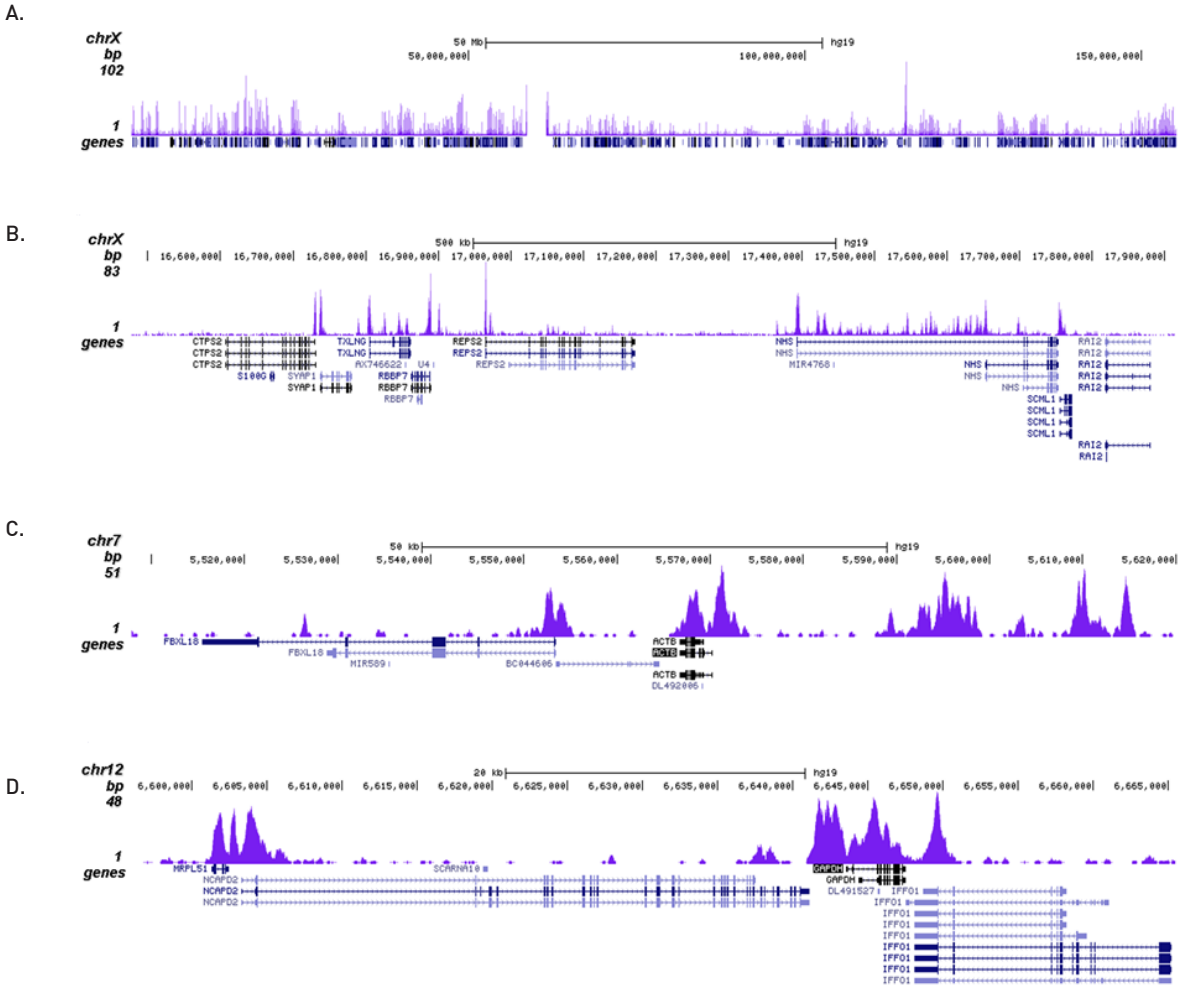

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K4me2

ChIP was performed on HeLa cells using 0.5 µg of the Diagenode antibody against H3K4me2 (cat. No. C15410035). The IP'd DNA was analysed on an Illumina Hiseq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence and a 1.5 Mb region of the human X-chromosome (figure 2A and 2B) and in 2 chromosomal regions surrounding the ACTB and GAPDH positive control genes (figure 2C and D, respectively).

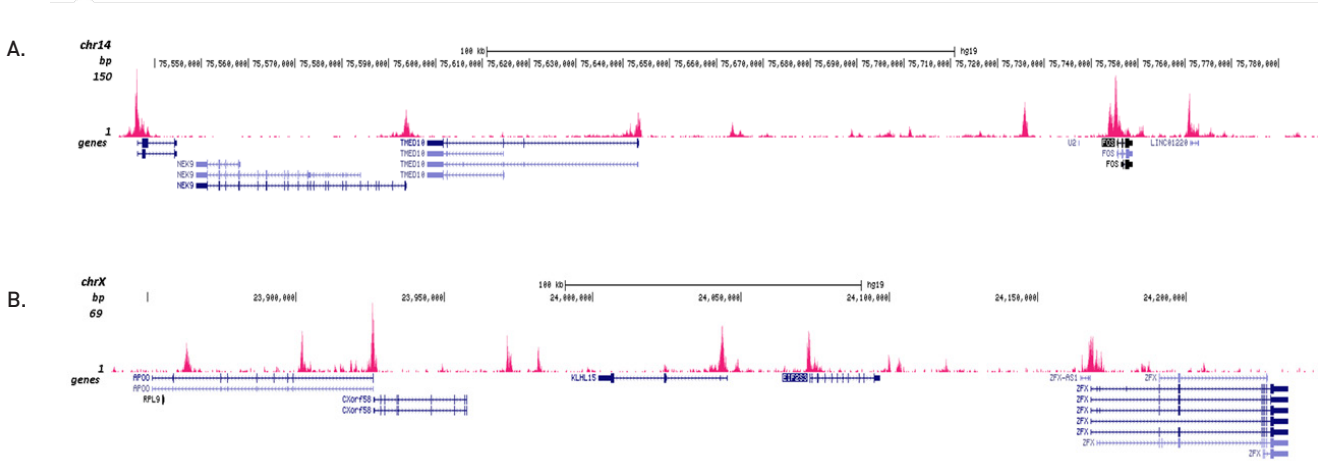

Figure 3. Cut&Tag results obtained with the Diagenode antibody directed against H3K4me2

CUT&TAG (Kaya-Okur, H.S., Nat Commun 10, 1930, 2019) was performed on 50,000 K562 cells using 0.5 µg of the Diagenode antibody against H3K4me2 (cat. No. C15410035) 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 in 2 genomic regions surrounding the FOS gene on chromosome 14 and the EIF2S3 gene on the X-chromosome (figure 3A and B, respectively).

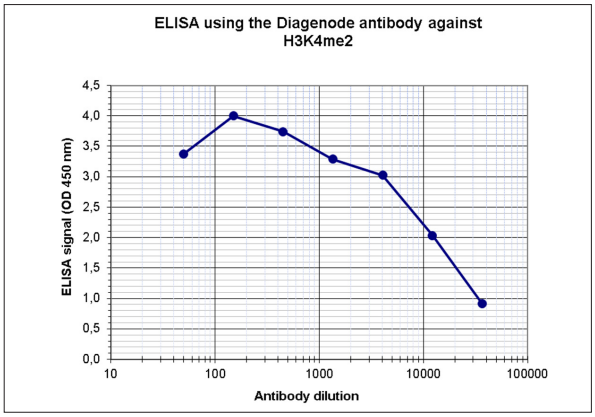

Figure 4. Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K4me2 (cat. No. C15410035) 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 4), the titer of the antibody was estimated to be 1:12,000.

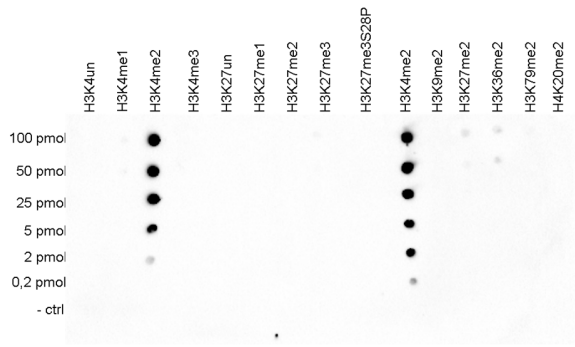

Figure 5. Cross reactivity test using the Diagenode antibody directed against H3K4me2

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K4me2 (cat. No. C15410035) 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 1:2,000. Figure 5 shows a high specificity of the antibody for the modification of interest.

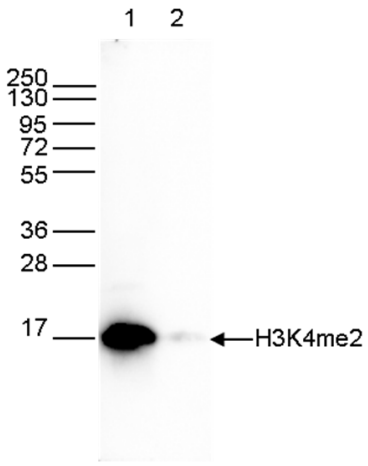

Figure 6. Western blot analysis using the Diagenode antibody directed against H3K4me2

Western blot was performed on whole cell extracts (25 µg, lane 1) and on 1 µg of recombinant histone H3 (lane 2) using the Diagenode antibody against H3K4me2 (cat. No. C15410035) 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.