diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K36me2 Antibody

Cat. No. C15310127

Type: Polyclonal	Specificity: Human, mouse, yeast: positive. Other species: not tested.
Size: 100 µl	Isotype: NA
Concentration: Not determined	Host: Rabbit
Lot No.: A239-001	Purity: Whole antiserum from rabbit.
Storage buffer: Whole antiserum from rabbit containing 0.05% azide	Storage conditions: 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: December, 2021

Description

Polyclonal antibody raised in rabbit against histone **H3 containing the dimethylated lysine 36 (H3K36me2)**, using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	0.5-1 µl/ChIP	Fig 1, 2
CUT&TAG	1 µg	Fig 3
ELISA	1:1,000	Fig 4
Dot Blotting Western	1:100,000	Fig 5
Blotting	1:1,000	Fig 6
Immunofluorescence	1:500	Fig 7

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5-10 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.

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Validation Data

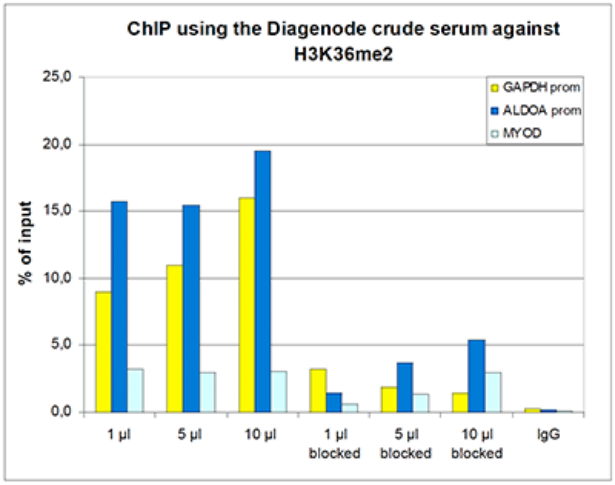

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K36me2

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K36me2 (Cat. No. C15310127) and optimized PCR primer sets for qPCR. ChIP was performed with the "LowCell# ChIP" kit (Cat. No. C01010070), using sheared chromatin from 10,000 cells. A titration of the antibody consisting of 1, 5, and 10 µl per ChIP experiment was analysed. Additionally, the same titration was analysed after incubation of the antibody with 5 nmol blocking peptide (Cat. No. C16000127) for 1 hour at room temperature. IgG (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoter of the active genes GAPDH and ALDOA and for the coding region of the myogenic differentiation gene (MYOD). Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

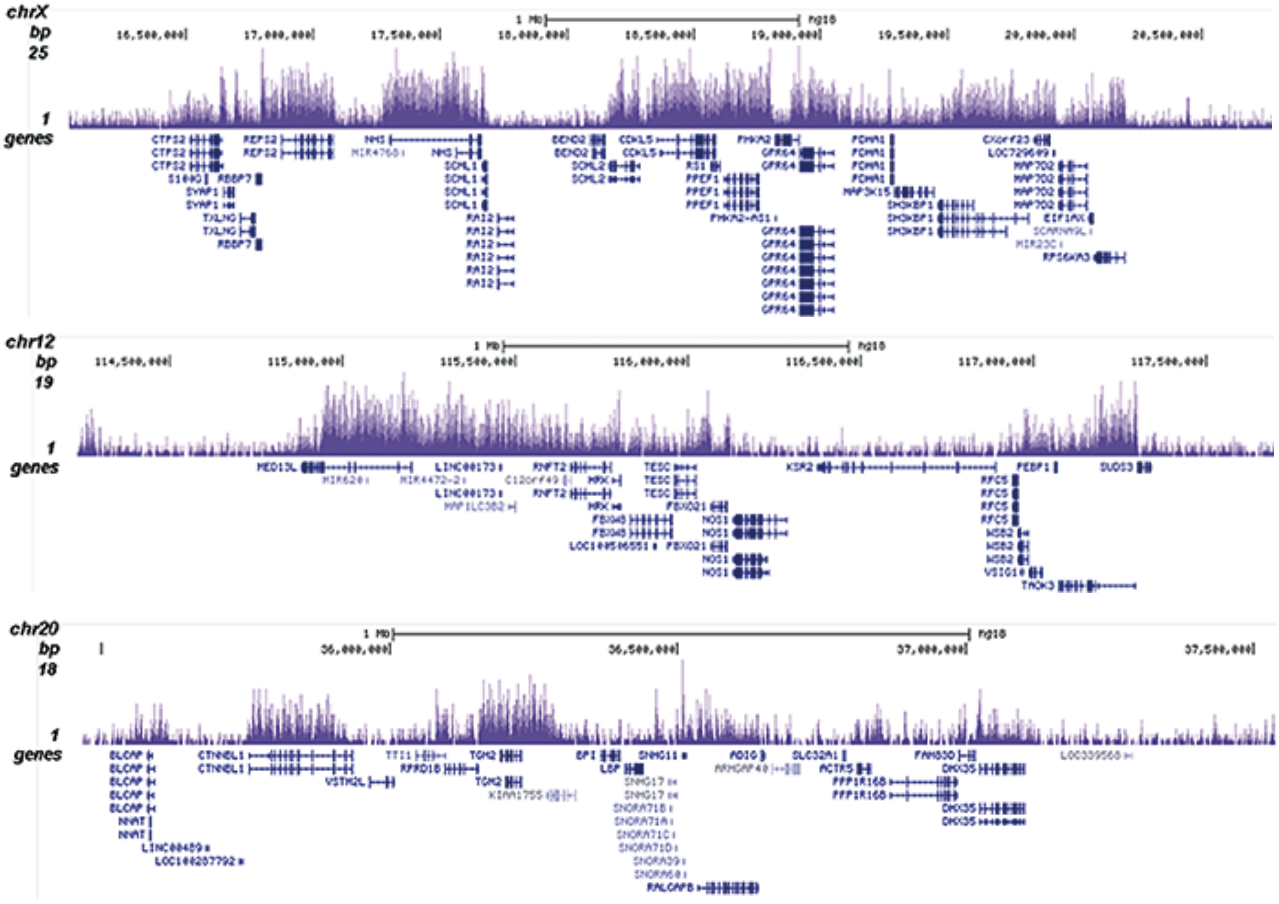

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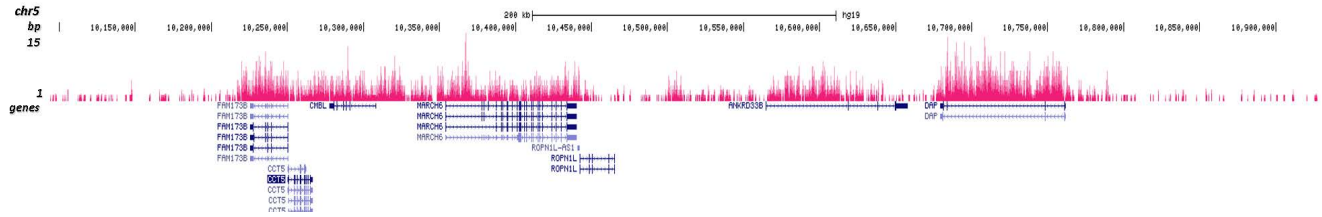
Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K36me2

ChIP was performed with 0.5 µl of the Diagenode antibody against H3K36me2 (Cat. No. C15310127) on sheared chromatin from 1 million HeLa cells using the "iDeal ChIP-seq" kit (Cat. No. C01010051). 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 signal distribution along 3 genomic regions of chromosome 20, 12 and X, respectively.

A.



B.

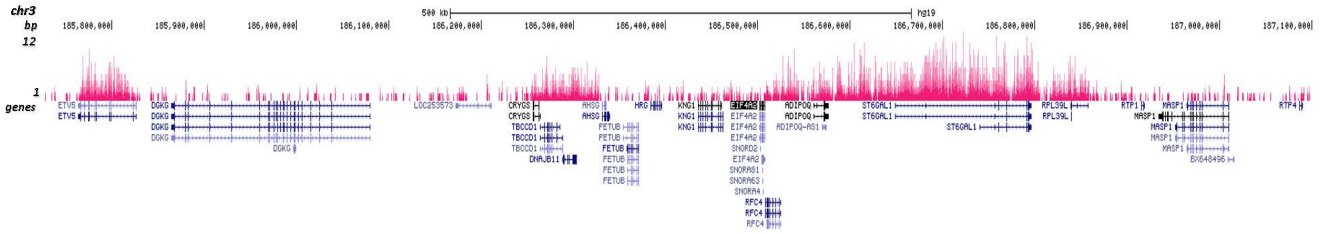


Figure 3. Cut&Tag results obtained with the Diagenode antibody directed against H3K36me2

CUT&TAG (Kaya-Okur, H.S., Nat Commun 10, 1930, 2019) was performed on 50,000 K562 cells using 1 µg of the Diagenode antibody against H3K36me2 (cat. No. C15310127) 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 MARCH6 gene on chromosome 5 and the EIF4A2 gene on chromosome 3 (figure 3A and B, respectively).

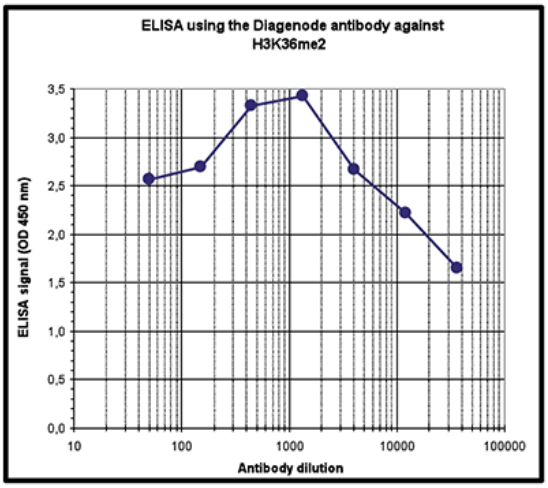


Figure 4. Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K36me2 (Cat. No. C15310127). 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 1:31,000.

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Figure 5. Cross reactivity test using the Diagenode antibody directed against H3K36me2

A dot blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K36me2 (Cat. No. C15310127) with peptides containing other modifications and unmodified sequences of histone H3. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:100,000. Figure 5 shows a high specificity of the antibody for the modification of interest.

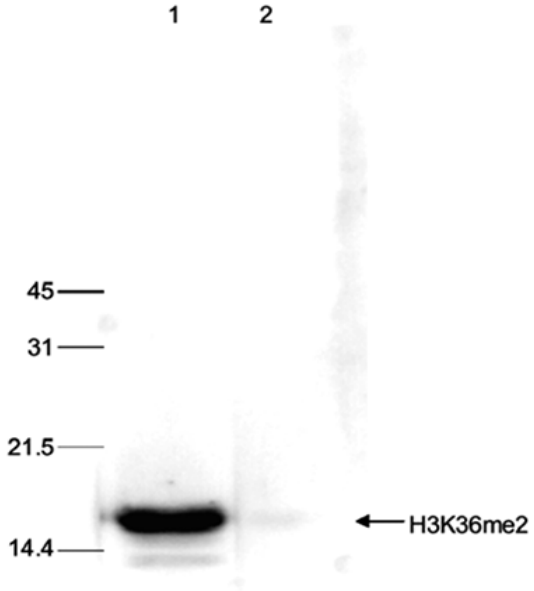

Figure 6. Western blot analysis using the Diagenode antibody directed against H3K36me2

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K36me2 (Cat. No. C15310127) 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. The result of the Western analysis with the antibody is shown in lane 1; lane 2 shows the same analysis after incubation of the antibody with 5 nmol blocking peptide (Cat. No. C16000127) for 1 hour at room temperature.

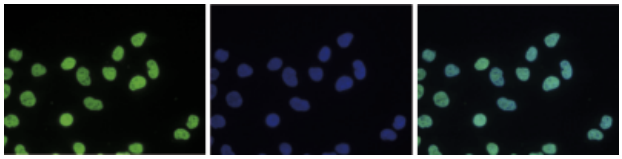

Figure 7. Immunofluorescence using the Diagenode antibody directed against H3K36me2

HeLa cells were stained with the Diagenode antibody against H3K36me2 (Cat. No. C15310127) 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 H3K36me2 antibody (left) diluted 1:500 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.

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