H3K4me3 polyclonal antibody

Cat. No. C15410030

Type: Polyclonal	Specificity: Human, mouse, Arabidopsis: positive. Other species: not tested.
Size: 50 µg	Isotype: NA
Concentration: 1.4 µg/µl	Source: Rabbit
Lot No.: 002	Purity: Affinity purified polyclonal antibody.
Storage buffer: PBS containing 0.05% azide and 0.05% ProClin 300.	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: May 3, 2018

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

Applications	Suggested dilution	References
ChIP *	1 µg/ChIP	Fig 1, 2
Dot Blotting	1:2,000	Fig 3
Western Blotting	1:500	Fig 4
Immunofluorescence	1:100	Fig 5

* 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. Trimethylation of histone H3K4 is associated with active promoters.

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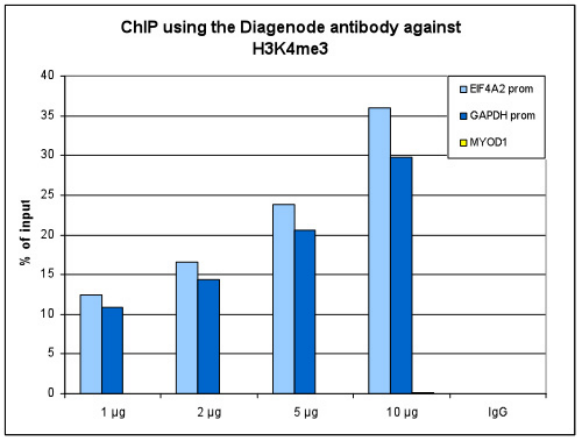
Validation data

A.



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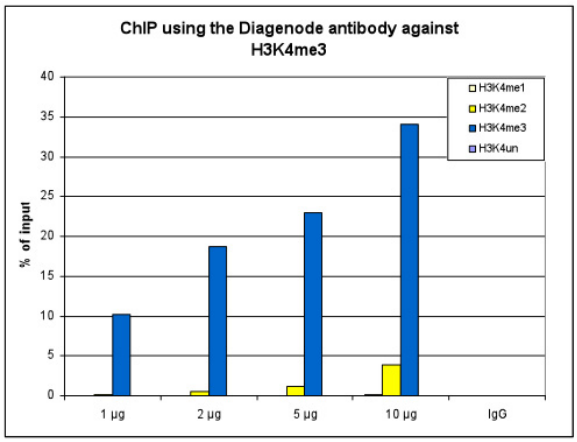
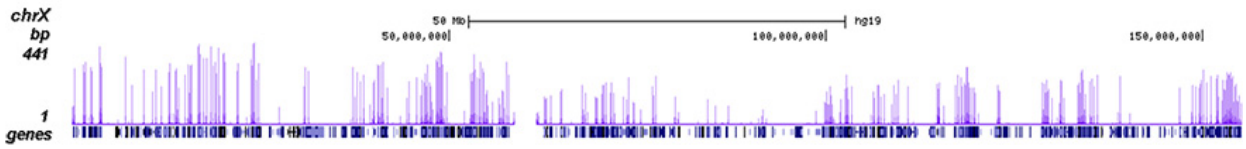


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4me3

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K4me3 (Cat. No. C15410030) and optimized PCR primer pairs for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (cat. No. C01010051), using sheared chromatin from 1 million cells. The chromatin was spiked with a panel of in vitro assembled nucleosomes, each containing a specific lysine methylation (SNAP-ChIP K-MetStat Panel, Epicypher). 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. **Figure 1A.** Quantitative PCR was performed with primers specific for the promoter of the active GAPDH and EIF4A2 genes, used as positive controls, and for the inactive MYOD1 gene, used as negative control. The graph 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 1B.** Recovery of the nucleosomes carrying the H3K4me1, H3K4me2, H3K4me3 modifications and the unmodified H3K4 as determined by qPCR. The figure clearly shows the antibody is very specific in ChIP for the H3K4me3 modification. At higher concentrations some H3K4me2 is also precipitated.

A.



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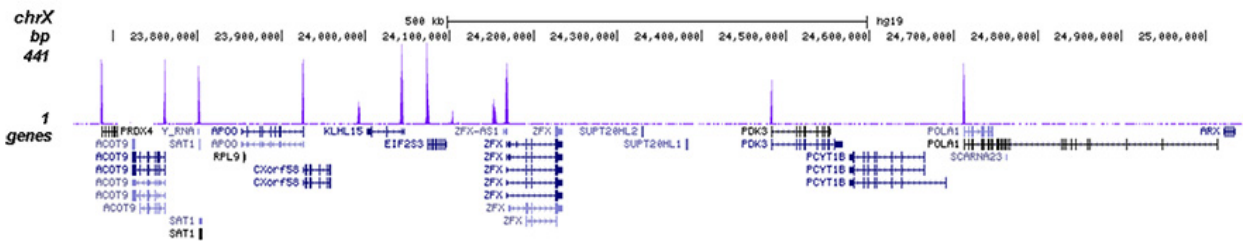

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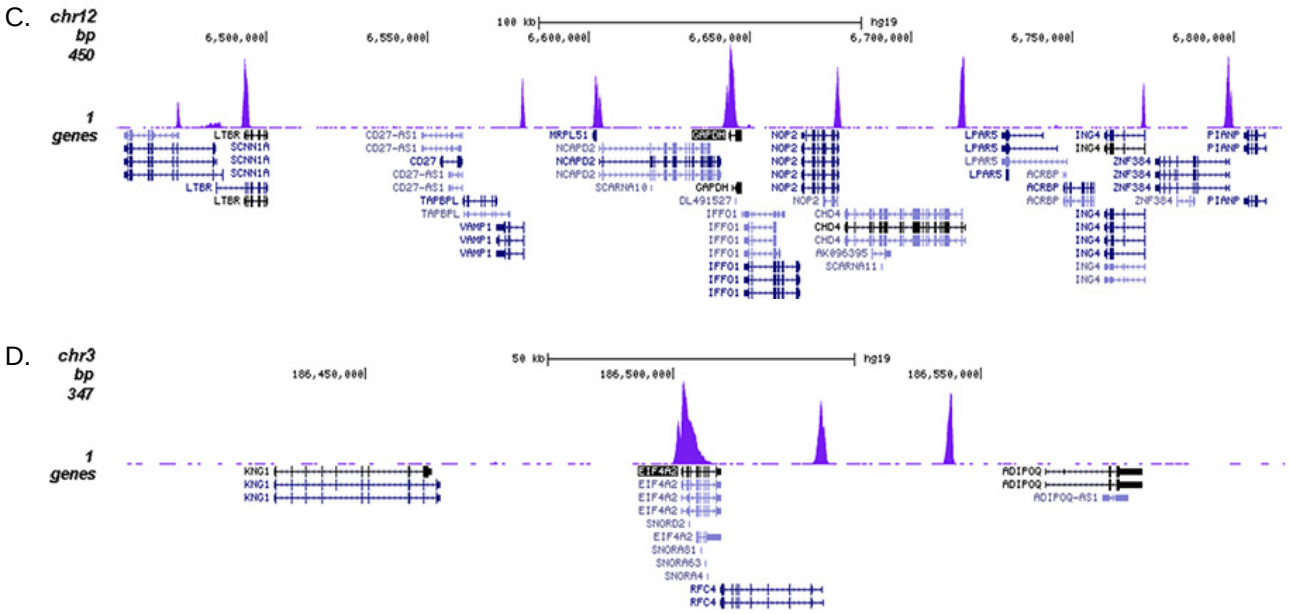

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K4me3

ChIP was performed on sheared chromatin from 1 million HeLa cells using 1 µg of the Diagenode antibody against H3K4me3 (Cat. No. C15410030) 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 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence and a 1.2 Mb region of the X-chromosome (figure 2A and B) and in two regions surrounding the GAPDH and EIF4A2 positive control genes, respectively (figure 2C and D). These results clearly show an enrichment of the H3K4 trimethylation at the promoters of active genes.

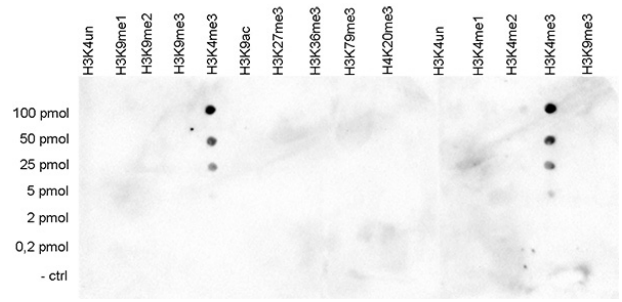

Figure 3. Cross reactivity test using the Diagenode antibody directed against H3K4me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K4me3 (Cat. No. C15410030) with peptides containing other modifications and unmodified sequences of histone H3 and H4. 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 3 shows a high specificity of the antibody for the modification of interest.

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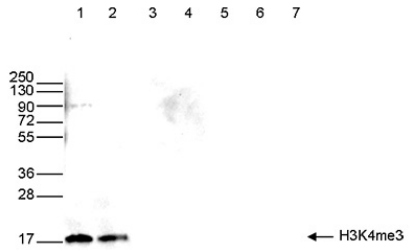

Figure 4. Western blot analysis using the Diagenode antibody directed against H3K4me3

Western blot was performed on whole cell (40 µg, lane 1) and histone extracts (15 µg, lane 2) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3.1, H3.3 and H4 (lane 3, 4, 5, 6 and 7, respectively) using the Diagenode antibody against H3K4me3 (Cat. No. C15410030). The antibody was diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is shown on the right, the marker (in kDa) is shown on the left.

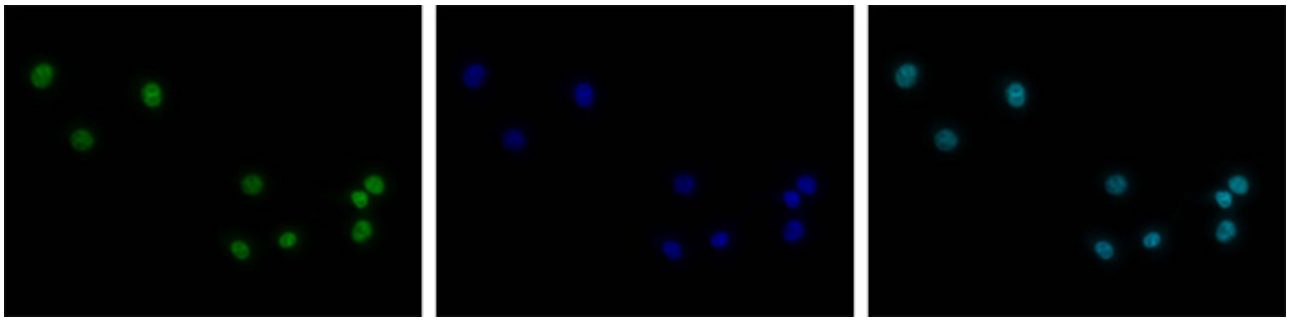

Figure 5. Immunofluorescence using the Diagenode antibody directed against H3K4me3

HeLa cells were stained with the Diagenode antibody against H3K4me3 (Cat. No. C15410030) 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 1:100 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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