

H3K4me1 polyclonal antibody

Cat. No. C15410037-50

Type: Polyclonal	Specificity: Human, mouse
Size: 50 µg	Isotype: NA
Concentration: 2.9 µg/µl	Host: Rabbit
Lot No.: A1657D	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: January 6, 2020

Description

Polyclonal antibody raised in rabbit against histone H3 containing the monomethylated lysine 4 (H3K4me1), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP *	1-2 µg/IP	Fig 1, 2
ELISA	1:500	Fig 3
Dot Blotting	1:10,000	Fig 4
Western Blotting	1:500	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.

Validation Data

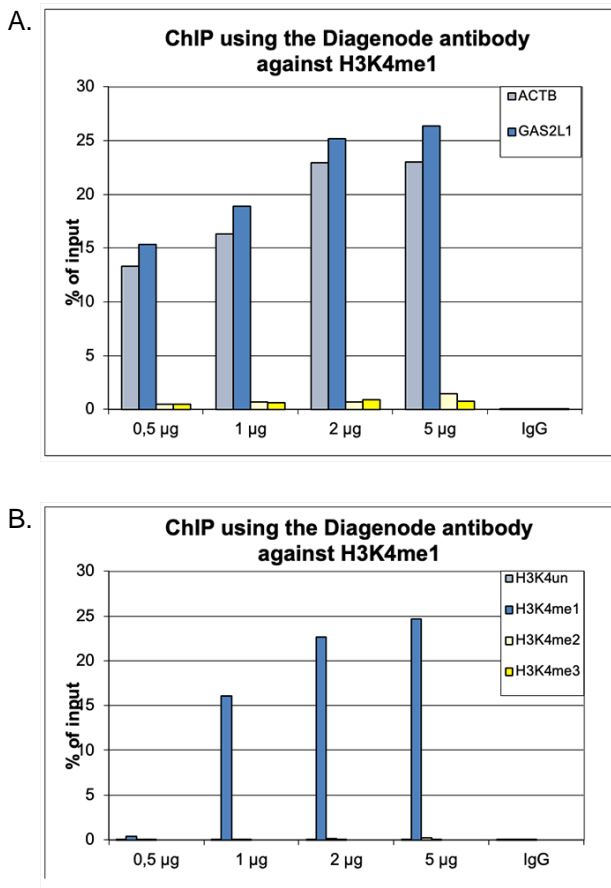
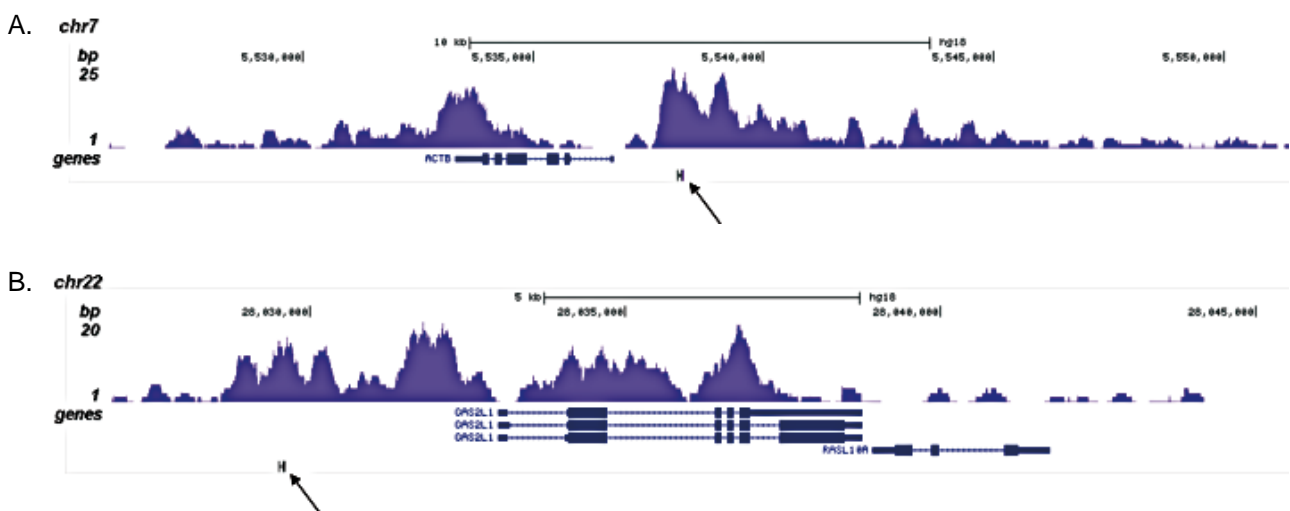


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4me1

ChIP was performed with the Diagenode antibody against H3K4me1 (cat. No. C15410037) on sheared chromatin from 500,000 HeLaS3 cells using the “iDeal ChIP-seq” kit (cat. No. C01010051). 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 of the antibody consisting of 0.5, 1, 2 and 5 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control.

Figure 1A. Quantitative PCR was performed with primers for a region surrounding the ACTB and GAS2L1 genes, used as positive controls, and for the promoters of the GAPDH and EIF4A2 genes, 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 1B.** Recovery of the nucleosomes carrying the H3K4me1, H3K4me2, H3K4me3, H3K9me1, H3K27me1, H3K36me1, H4K20me1 and the unmodified H3K4 as determined by qPCR. The figure clearly shows the antibody is very specific in ChIP for the H3K4me1 modification.



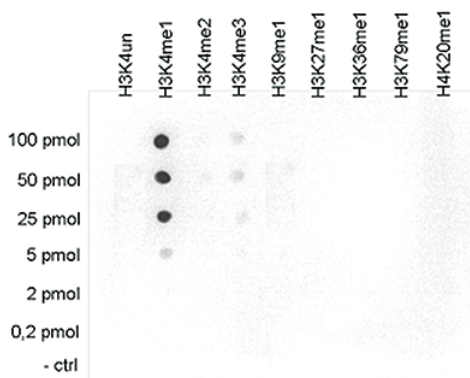


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K4me1

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K4me1 (Cat. No. C15410037) with peptides containing other modifications or 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 1:10,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

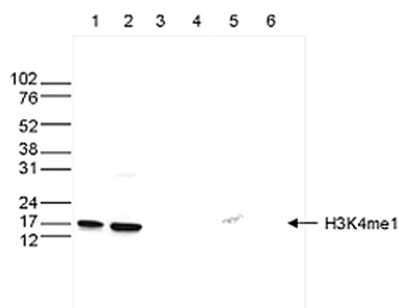


Figure 5. Western blot analysis using the Diagenode antibody directed against H3K4me1

Western blot was performed on whole cell (25 µg, lane 1) and histone extracts (15 µg, lane 2) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the Diagenode antibody against H3K4me1 (Cat. No. C15410037). 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 6. Immunofluorescence using the Diagenode antibody directed against H3K4me1

HeLa cells were stained with the Diagenode antibody against H3K4me1 (cat. C15410037) 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 labeled with the H3K4me1 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.