diagenode
A Hologic Company

ChIP-seq grade

TECHNICAL DATASHEET

H3K4me1 antibody

Cat. No. C15410037

Type: Polyclonal

Source: Rabbit

Lot: A1657D

Size: 50 µg

Concentration: 2.9 µg/µl

Specificity: Human, mouse, pig: 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 and 0.05% ProClin 300.

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, acetylated at lysine 27 (H3K27ac), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	1 - 2 µg per ChIP	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.

Diagenode sa. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue du Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode LLC. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

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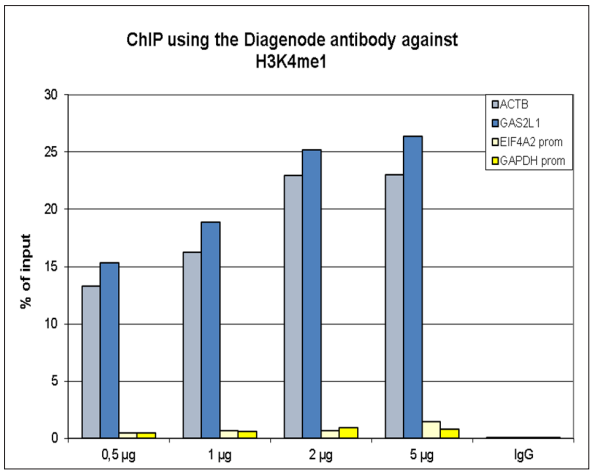
Results

A.



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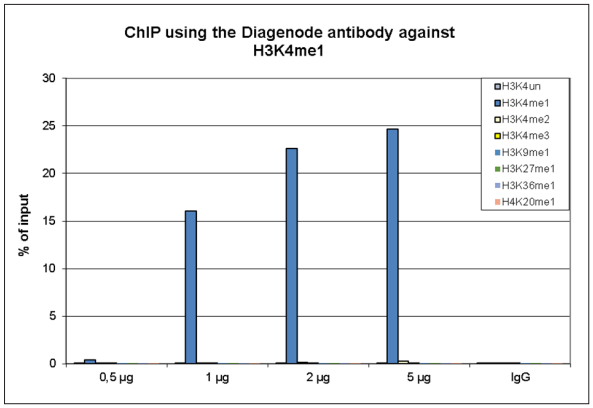


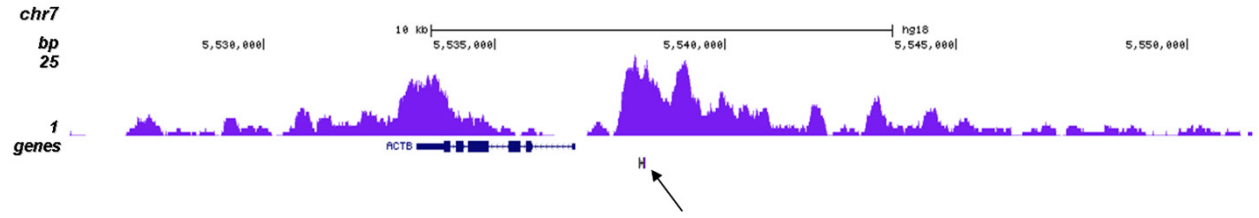
Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4me1

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.

A.



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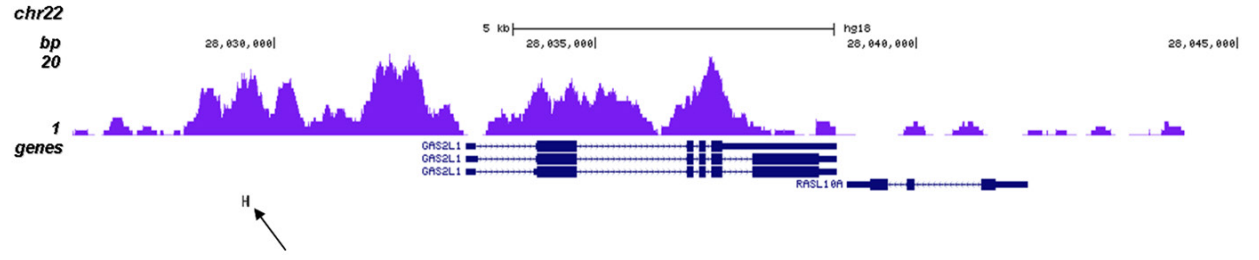
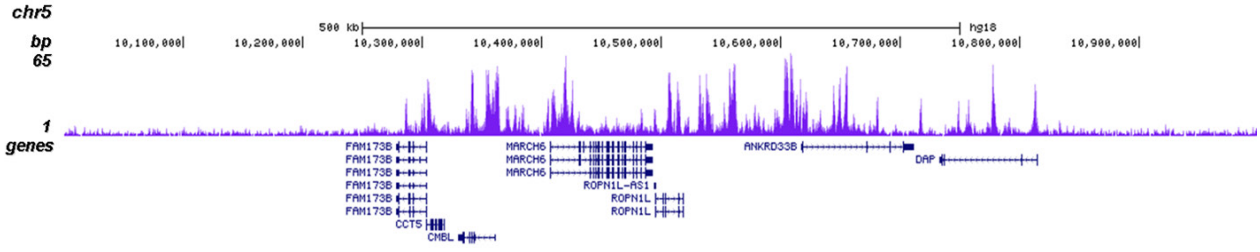


C.



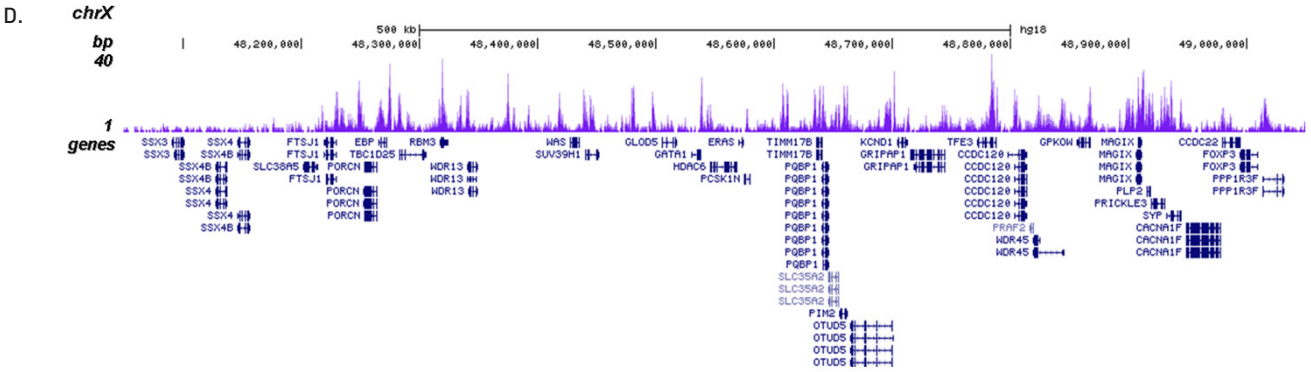


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K4me1

ChIP was performed as described above with 1 µg of the Diagenode antibody against H3K4me1 (cat. No. C15410037). 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 2A and B show the enrichment in chromosomal regions surrounding the ACTB and GAS2L1 positive control genes. The position of the amplicon used in the qPCR validation is indicated by an arrow. Figure 2C and D show the H3K4me1 signal in two 1 Mb regions of chromosome 5 and X, respectively.

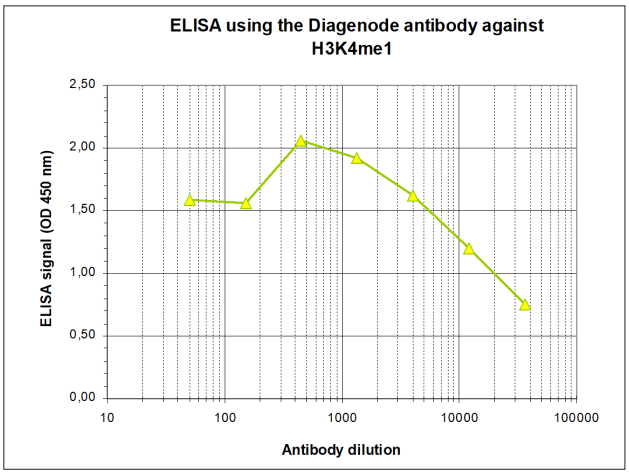


Figure 3. Determination of the antibody titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K4me1 (cat. No. C15410037) 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 3), the titer of the antibody was estimated to be 1:20,100.

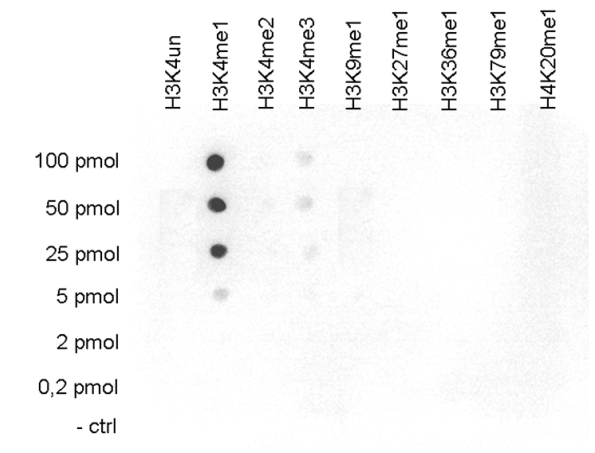


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K4me1

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. 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.

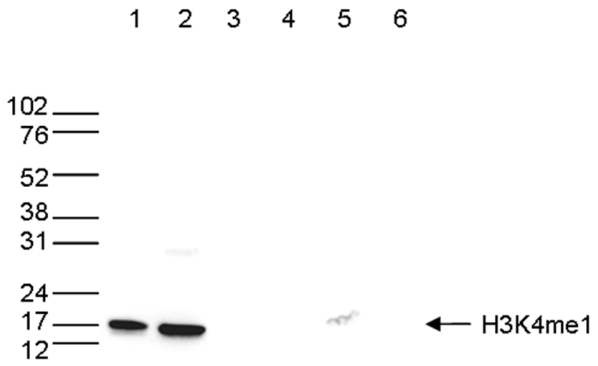


Figure 5. Western blot analysis using the Diagenode antibody directed against H3K4me1

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.

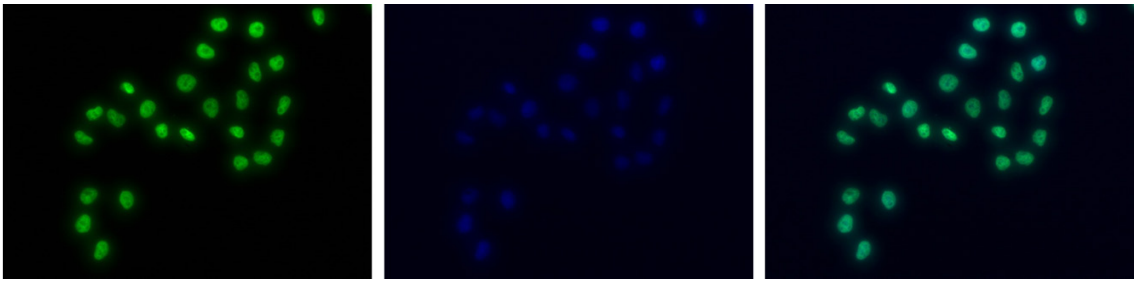


Figure 6. Immunofluorescence using the Diagenode antibody directed against H3K4me1

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.