diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K4me3 antibody

Cat. No. C15410003

Type: Polyclonal **ChIP grade/ChIP-seq grade**
Source: Rabbit
Lot: A8034D
Size: 50 µg
Concentration: 1.3 µg/µl

Specificity: Human, mouse, pig, zebrafish, trout, Daphnia, Arabidopsis, rice, tomato, maize, poplar, silena latifolia, wide range expected.
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 the region of histone H3 containing the trimethylated lysine 4 (H3K4me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	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
Immunofluorescence	1:200	Fig 7

*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. Methylation of histone H3K4 is associated with activation of gene transcription.

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

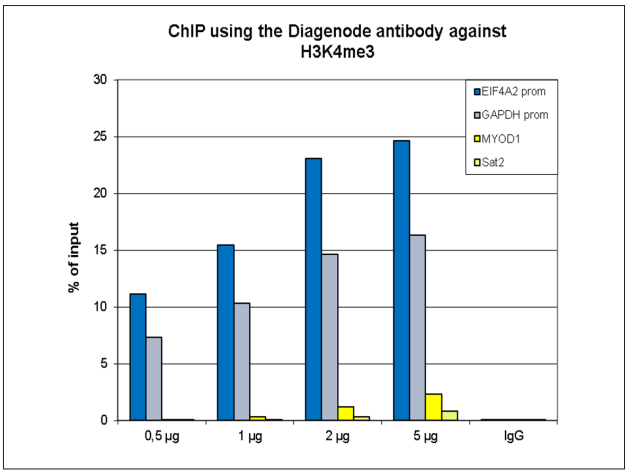

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K4me3

ChIP assays were performed using human K562 cells, the Diagenode antibody against H3K4me3 (cat. No. C15410003) and optimized PCR primer pairs for qPCR. ChIP was performed with the iDeal ChIP-seq kit (cat. No. C01010051), using sheared chromatin from 500,000 cells. A titration consisting of 0.5, 1, 2 and 5 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoter of the active genes GAPDH and EIF4A2, used as positive controls, and for the inactive MYOD1 gene and the Sat2 satellite repeat, used as negative controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

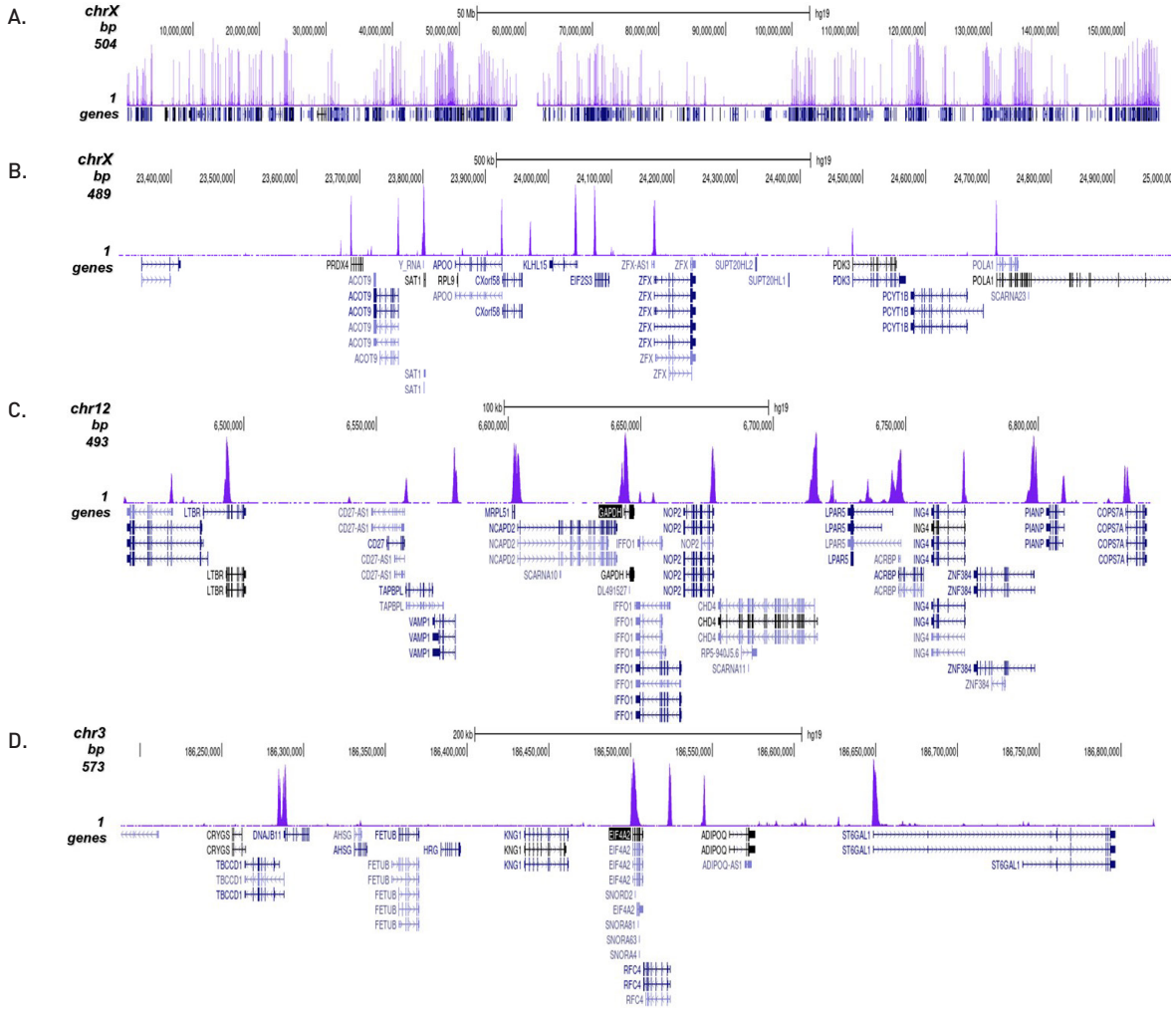

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K4me3

ChIP was performed on sheared chromatin from 1 million HeLa cells using 1 µg of the Diagenode antibody against H3K4me3 (cat. No. C15410003) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. 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 1.6 Mb region of the human X-chromosome (figure 2A and B) and in two regions surrounding the GAPDH and EIF4A2 positive control genes, respectively (figure 2C and D).

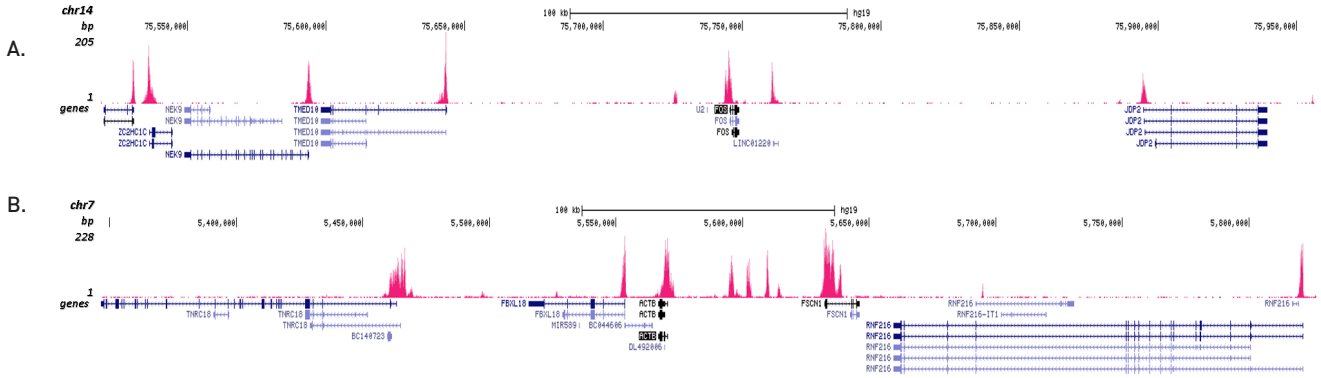

Figure 3. Cut&Tag results obtained with the Diagenode antibody directed against H3K4me3

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 H3K4me3 (cat. No. C15410003) 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 ACTB gene on chromosome 7 (figure 3A and B, respectively).

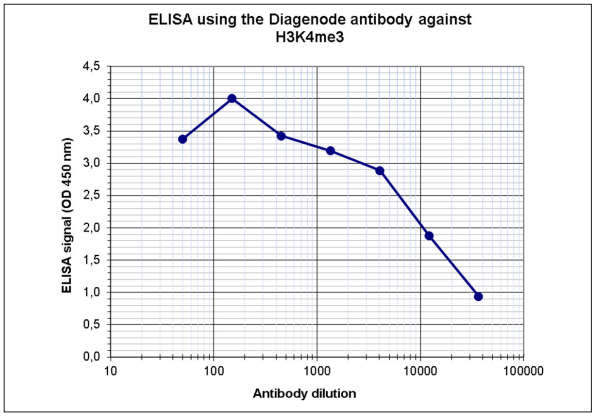

Figure 4. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against H3K4me3 (cat. No. C15410003). 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 1:11,000.

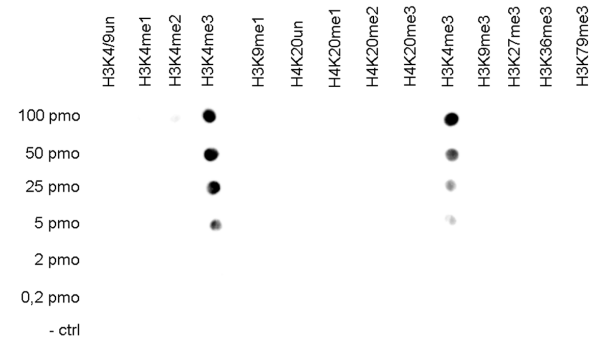

Figure 5. Cross reactivity tests using the Diagenode antibody directed against H3K4me3

To test the cross reactivity of the Diagenode antibody against H3K4me3 (cat. No. C15410003), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K4. 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.

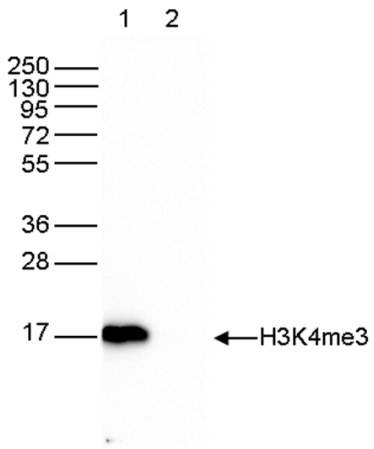

Figure 6. Western blot analysis using the Diagenode antibody directed against H3K4me3

Western blot was performed on whole cell extracts (40 µg, lane 1) from HeLa cells, and on 1 µg of recombinant histone H3 (lane 2) using the Diagenode antibody against H3K4me3 (cat. No. C15410003). The antibody was 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.

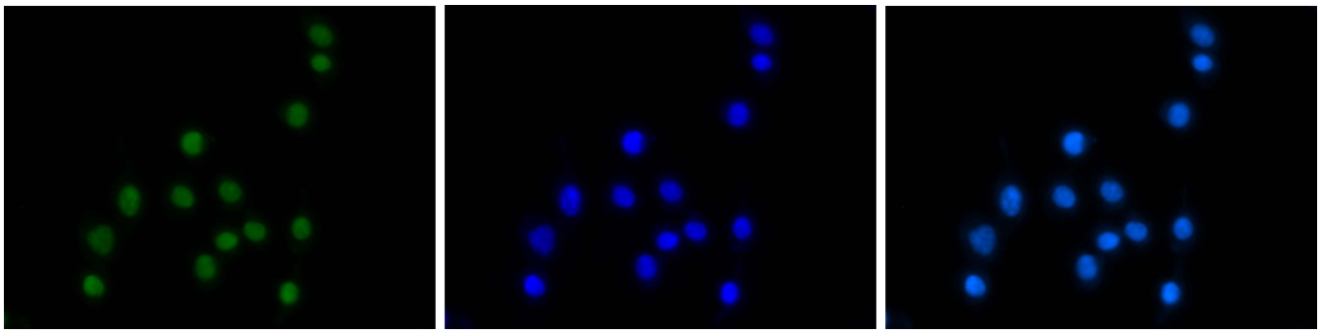

Figure 7. Immunofluorescence using the Diagenode antibody directed against H3K4me3

HeLa cells were stained with the Diagenode antibody against H3K4me3 (cat. No. C15410003) and with DAPI. Cells were fixed with 4% formaldehyde for 20' and blocked with PBS/TX-100 containing 5% normal goat serum. The cells were immunofluorescently labelled with the H3K4me3 antibody (left) diluted 1:200 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa568 or with DAPI (middle), which specifically labels DNA. The right picture shows a merge of both stainings.