

H3K9me3 antibody

Cat. No. C15410193

Lot:	A2814P
Size:	10 µg / 50 µg
Type:	Polyclonal, ChIP/ChIP-seq-grade
Isotype:	NA
Source:	Rabbit
Concentration:	2.1 µg/µl

Specificity:	Human, mouse, zebrafish, trout, Daphnia, Arabidopsis, Drosophila, Silene latifolia: positive. Other species: not tested.
Purity:	Affinity purified polyclonal antibody
Storage buffer:	PBS containing 0.05% azide.

Storage: Store at -20 °C. For long-term 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.

Description: Polyclonal antibody raised in rabbit against the region of histone H3 containing the trimethylated lysine 9 (H3K9me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	0.5–1 µg per ChIP	Fig 1, 2
ELISA	1:1,000–1:10,000	Fig 3
Dot blotting	1:2,000	Fig 4
Western blotting	1:2,000	Fig 5

*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 the 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 are dynamically regulated by histone methyltransferases and histone demethylases, respectively. Trimethylation of histone H3K9 is associated with inactive regions of chromatin.

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@hologic.com
support.diagenode@hologic.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

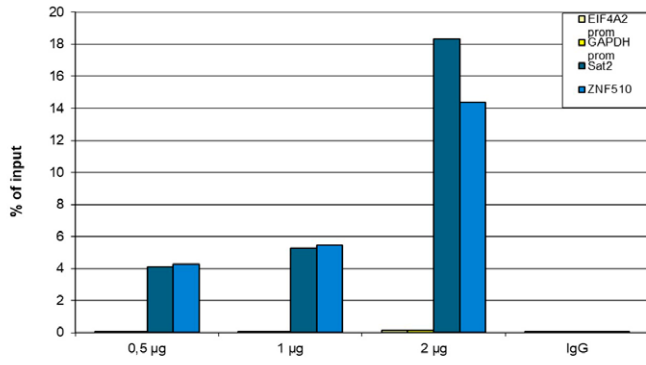


Figure 1: ChIP results obtained with the antibody directed against H3K9me3

ChIP was performed with the antibody against H3K9me3 (cat. no. C15410193) on sheared chromatin from 500,000 K562 cells using the iDeal ChIP-seq kit (cat. no. C01010051). A titration of the antibody consisting of 0.5, 1, and 2 µg per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers for the ZNF510 gene and the Sat2 satellite repeat, used as positive controls, and for the EIF4A2 and GAPDH promoters, 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).

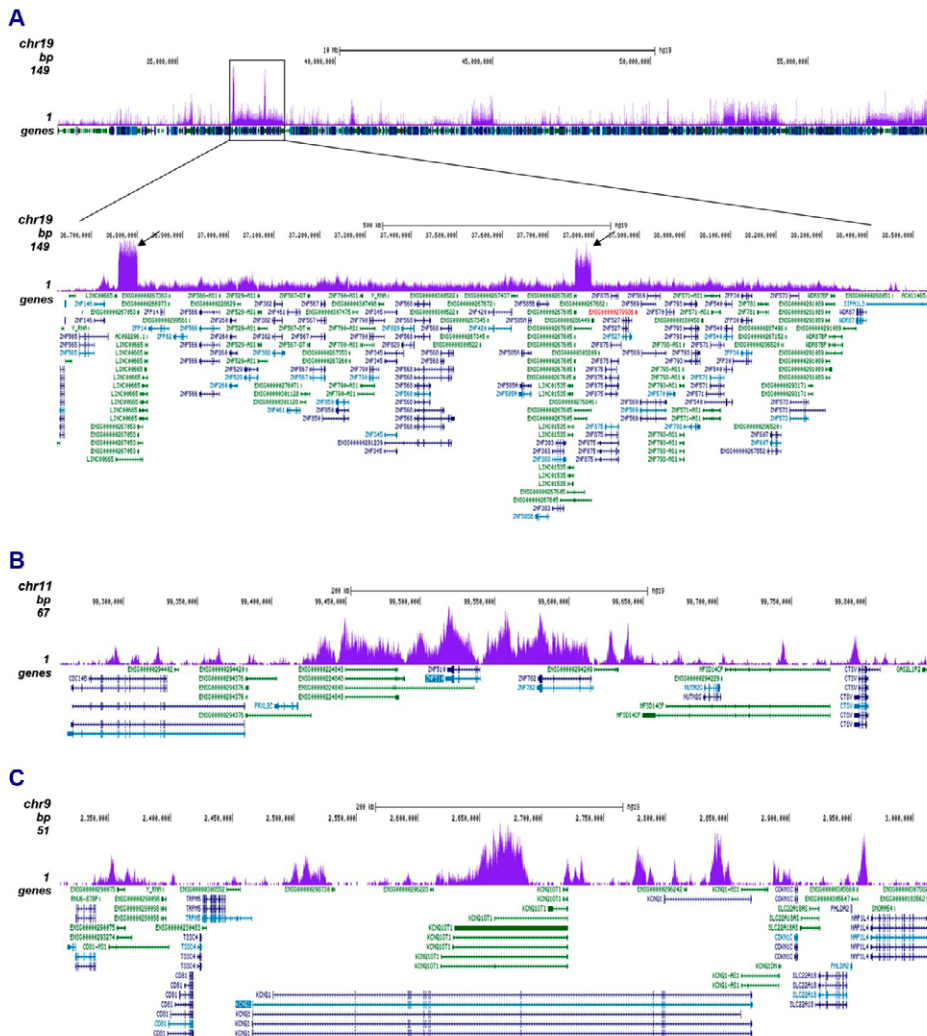


Figure 2: ChIP-seq results obtained with the antibody directed against H3K9me3

ChIP was performed with 1 µg of the antibody against H3K9me3 (cat. no. C15410193) on sheared chromatin from 500,000 K562 cells using the iDeal ChIP-seq kit as described above. The IP'd DNA was subsequently analyzed on an Illumina HiSeq4000. 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 2A shows the signal distribution along the long arm of chromosome 19 and a zoom-in to an enriched region containing several ZNF repeat genes. The arrows indicate two satellite repeat regions which exhibit a stronger signal. Figures 2B and 2C show the enrichment along the ZNF510 positive control target and at the KCNQ1 imprinted gene.

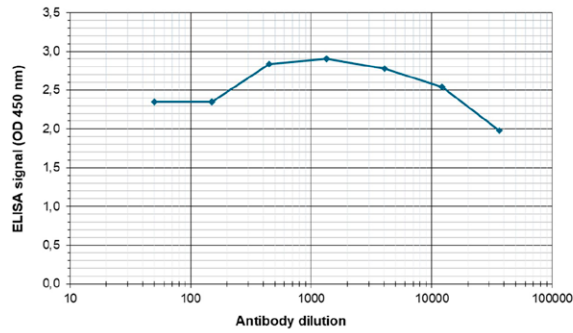


Figure 3: Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody directed against human H3K9me3 (cat. no. C15410193) 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 1:183,000.

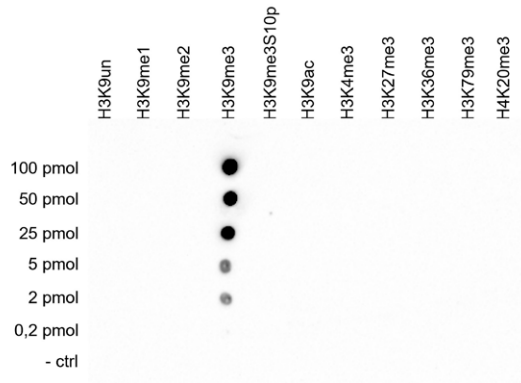


Figure 4: Cross-reactivity tests using the antibody directed against H3K9me3

A Dot Blot analysis was performed to test the cross-reactivity of the antibody against H3K9me3 (cat. no. C15410193) with peptides containing other modifications of histone H3 and H4 and the unmodified sequence 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 1:2,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

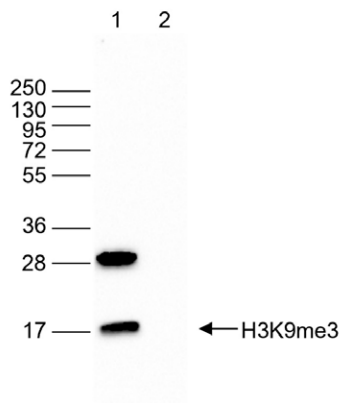


Figure 5: Western blot analysis using the antibody directed against H3K9me3

Western blot was performed on 40 µg of whole cell extracts from HeLa cells (lane 1) and on 1 µg of recombinant histone H3 (lane 2) using the antibody against H3K9me3 (cat. no. C15410193). The antibody was diluted 1:2,000 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.