TECHNICAL DATASHEET

LOT: 003

HOLOGIC® Diagenode

H3K9me3 antibody

Cat. No. C15200146

Lot:	003
Size:	10 µg / 50 µg
Type:	Monoclonal, **ChIP-grade**, **ChIP-seq grade**, **CUT&Tag-grade**
Isotype:	IgG1
Source:	Mouse
Concentration:	1.7 µg/µl

Specificity:	Human, mouse, fungi: positive Other species: not tested
Purity:	Protein A purified monoclonal antibody
Storage buffer:	PBS containing 0.05% azide.

Storage: 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.

Description: Monoclonal antibody raised in mouse against histone H3 trimethylated at 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
CUT&Tag	1 µg	Fig 3
ELISA	1:100	Fig 4
Dot blotting	1:100,000	Fig 5
Western blotting	1:1,000	Fig 6
Immunofliorescence	1:500	Fig 7

*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 present in the chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been highly conserved during evolution. Histones pack DNA into tight masses of chromatin. Two core histones from each of the class (H2A, H2B, H3, and H4) assemble into an octamer, which is wrapped by 146 base pairs of DNA to form one nucleosome. Histone tails undergo numerous post-translational modifications, which either directly or indirectly alter chromatin structure to facilitate transcriptional activation, 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 methyl transferases and histone demethylases, respectively.

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

Last update: December, 2025

Results

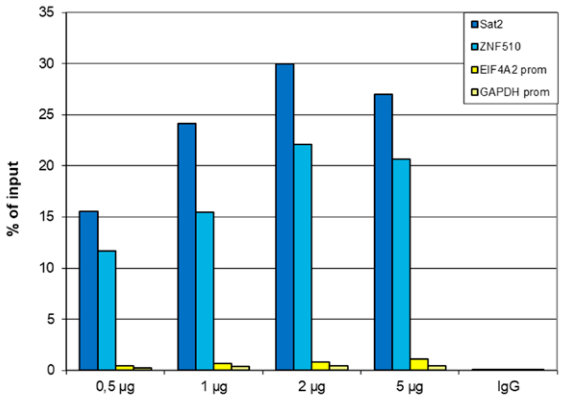

Figure 1: ChIP results obtained with the monoclonal antibody directed against H3K9me3

ChIP assays were performed on human HeLa cells using the monoclonal antibody against H3K9me3 (cat. No. C15200146). 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. qPCR was performed with primers for the promoters of the active EIF4A2 and GAPDH genes (used as negative controls) and for the ZNF510 gene and the Sat2 satellite repeat region (used as positive controls). Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

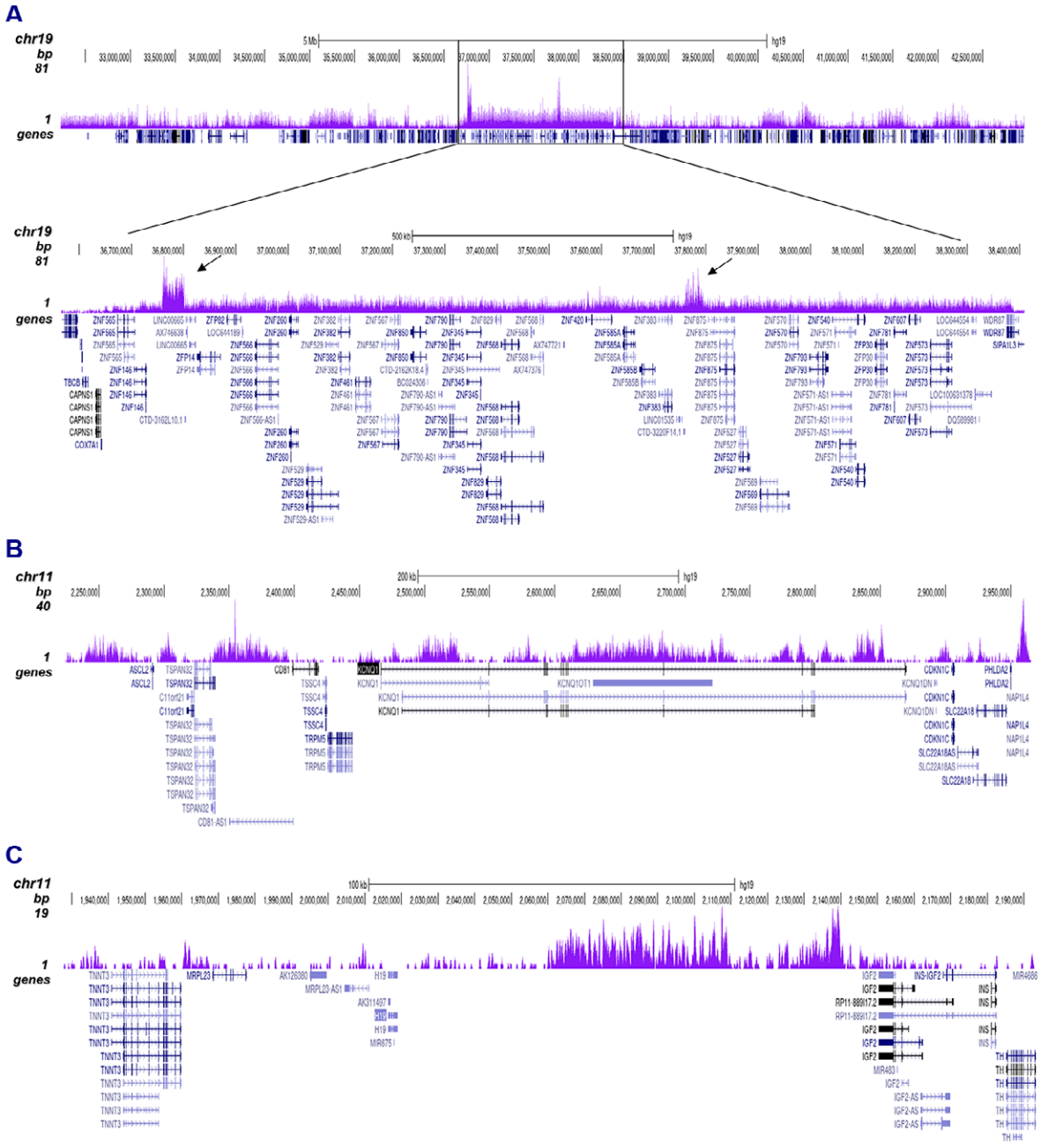

Figure 2: ChIP-seq results obtained with the antibody directed against H3K9me3

ChIP was performed with 0.5 µg of the antibody against H3K9me3 (cat. No. C15200146) on sheared chromatin from 500,000 HeLa cells using the iDeal ChIP-seq kit as described above. The IP'd DNA was subsequently analyzed 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 2A shows the signal distribution along the long arm of chromosome 19 and a zoom-in on an enriched region containing several ZNF repeat genes. The arrows indicate two satellite repeat regions that exhibit a stronger signal. Figures 2B and 2C show the enrichment at the H19 and KCNQ1 imprinted genes.

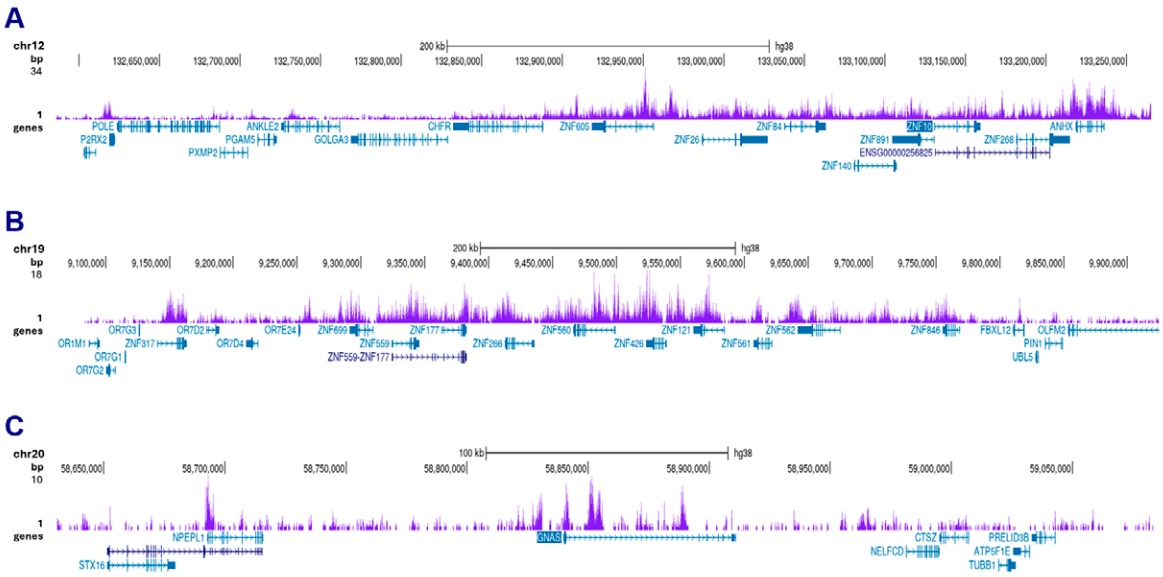

Figure 3: Cut&Tag results obtained with the antibody directed against H3K9me3

Cut&Tag was performed on 50,000 K562 cells using 1 µg of the antibody against H3K9me3 (cat. No. C15200146), the pA-Tn5 transposase (C01070001), and the iDeal Cut&Tag kit (cat. No. C01070021). The libraries were subsequently analyzed on an Illumina NovaSeq sequencer (2x50 bp paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg38) using the BWA algorithm. Figure 3 shows the peak distribution in two genomic regions on chromosomes 12 and 19 (each containing several ZNF repeat genes) and on chromosome 20 surrounding the GNAS imprinting control region (Figure 3A, B and C, respectively).

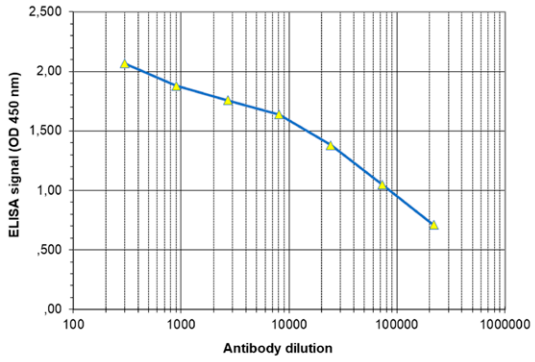

Figure 4: Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody directed against H3K9me3 (cat. No. C15200146) 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 4), the titer of the antibody was estimated to be 1:14,500.

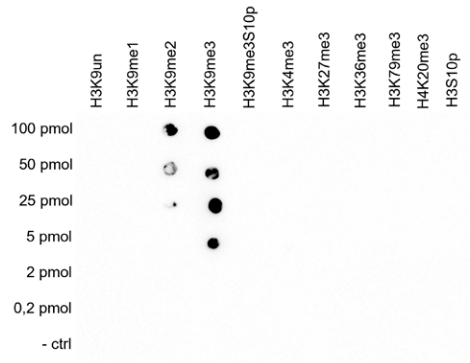

Figure 5: Cross reactivity test using the monoclonal antibody directed against H3K9me3

A Dot Blot analysis was performed to test the cross-reactivity of the monoclonal antibody against H3K9me3 (cat. No. C15200146) with peptides containing different modifications of histone H3 or H4, as well as the unmodified H3K9 sequence. One hundred to 0.2 pmol of peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:100,000. Figure 5 shows high specificity of the antibody for the modification of interest, with some cross-reactivity to the H3K9me2 peptide.

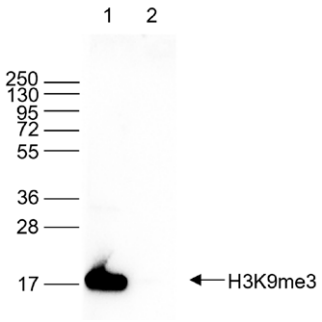

Figure 6: Western blot analysis using the monoclonal antibody directed against H3K9me3

Western blot was performed on histone extracts (15 µg, lane 1) from HeLa cells, and on 1 µg of recombinant histone H3 (lane 2) using the monoclonal antibody against H3K9me3 (cat. No. C15200146). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left; the position of the protein is indicated on the right.

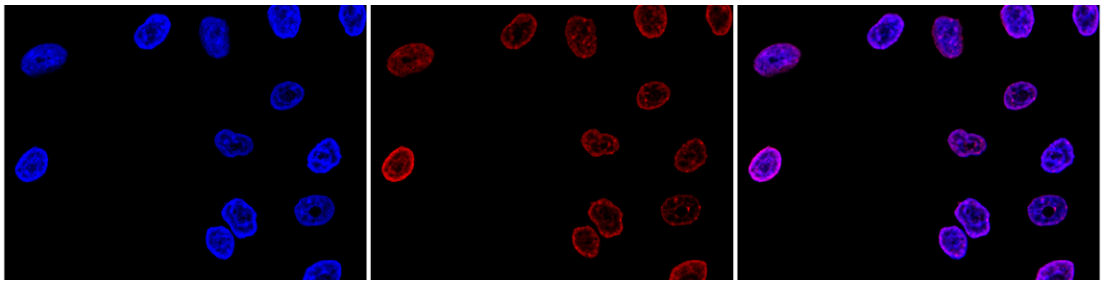

Figure 7: Immunofluorescence using the monoclonal antibody directed against H3K9me3

HeLa cells were stained with the antibody against H3K9me3 (cat. No. C15200146) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labeled with the H3K9me3 antibody (middle), diluted 1:500 in blocking solution, followed by an anti-mouse antibody conjugated to Alexa594. The left panel shows staining of the nuclei with DAPI. A merge of both stains is shown on the right.