

## H3K9me3 antibody

**Cat. No.** C15200146

**Type:** Monoclonal ChIP-grade, ChIP-seq grade

**Isotype** IgG1

**Source:** Mouse

**Lot:** 003

**Size:** 10 µg / 50 µg

**Concentration:** 1.7 µg/µl

**Specificity:** Human, mouse, fungi: positive  
Other species: not tested

**Purity:** Protein A purified monoclonal 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:** 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
ELISA	1:100	Fig 3
Dot blotting	1:100,000	Fig 4
Western blotting	1:1,000	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 present in 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 is dynamically regulated by respectively histone methyl transferases and histone demethylases.

#### Diagenode sa. BELGIUM | EUROPE

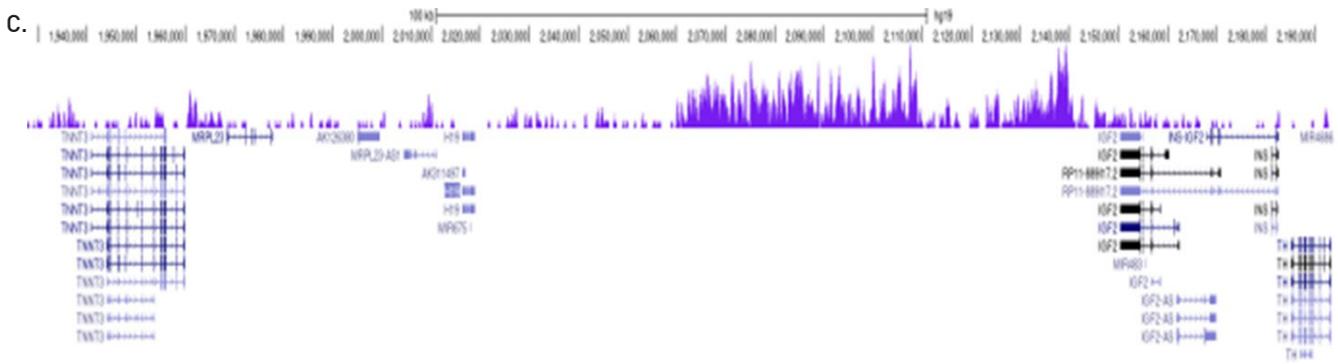
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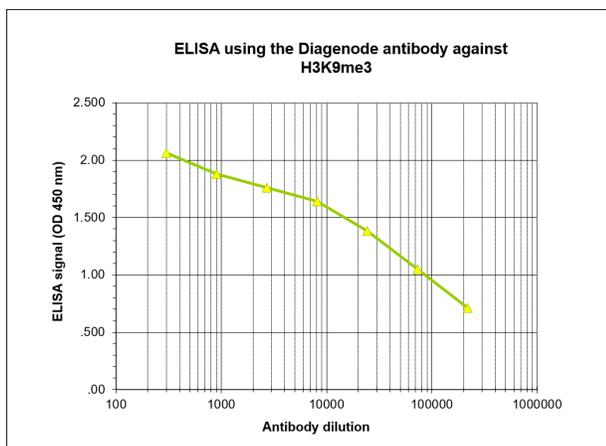
Last update: January, 2024





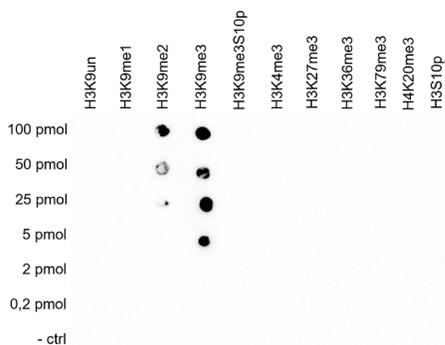
**Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K9me3**

ChIP was performed with 0.5 µg of the Diagenode 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 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 2A shows the signal distribution along the long arm of chromosome 19 and a zoomin 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 at the H19 and KCNQ1 imprinted genes.



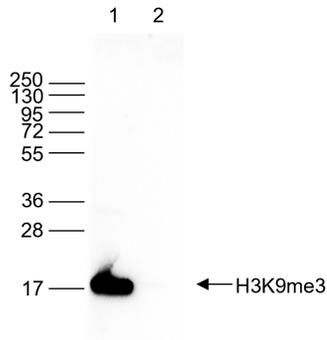
**Figure 3. Determination of the antibody titer**

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody directed against 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 3), the titer of the antibody was estimated to be 1:14,500.



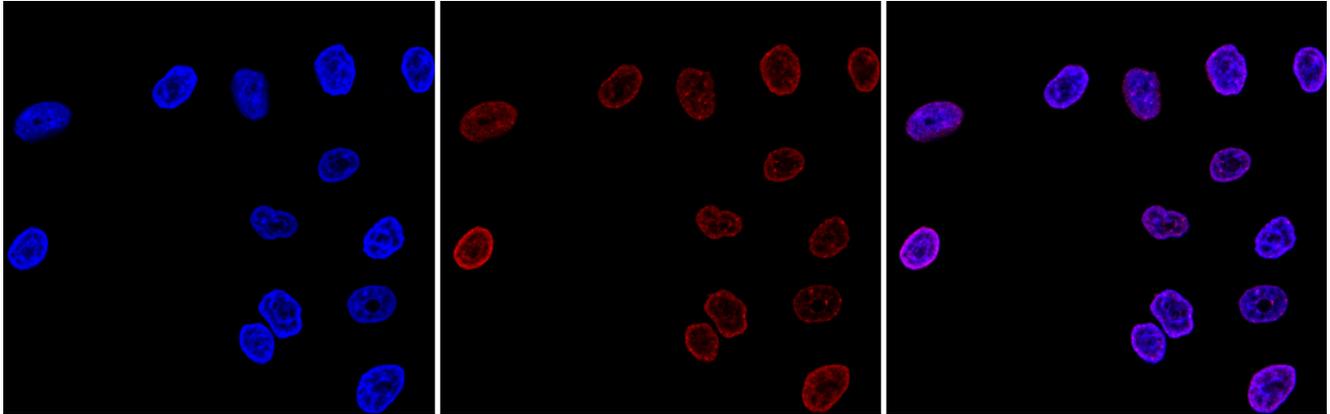
**Figure 4. Cross reactivity test using the Diagenode monoclonal antibody directed against H3K9me3**

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode monoclonal antibody against H3K9me3 (cat. No. C15200146) with peptides containing different modifications of histone H3 or H4 and 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 4 shows a high specificity of the antibody for the modification of interest, with some cross reaction with the H3K9me2 peptide.



**Figure 5. Western blot analysis using the Diagenode monoclonal antibody directed against H3K9me3**

Western blot was performed on histone extracts (15  $\mu$ g, lane 1) from HeLa cells, and on 1  $\mu$ g of recombinant histone H3 (lane 2) using the Diagenode 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 6. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K9me3**

HeLa cells were stained with the Diagenode 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 labelled 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 stainings is shown on the right.