diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K9me3 monoclonal antibody

Cat. No. C15200146
Type: Monoclonal **ChIP-grade**
Isotype: IgG1
Source: Human, mouse, fungi: positive
Lot #: 002
Size: 50 µg/ 28 µl
Concentration: 1.8 µg/µl

Specificity: Human, mouse, fungi: positive
Other species: not tested
Purity: Protein A purified monoclonal antibody in 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

	Suggested dilution	Results
ChIP*	1 µg/ChIP	Fig 1
Dot blotting	1:100,000	Fig 2
Western blotting	1:1,000	Fig 3
IF	1:500	Fig 4

* 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. Methylation of histone H3K9 is associated with gene repression.

Results

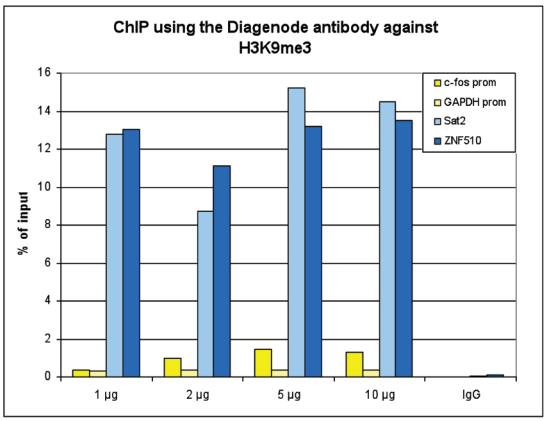

Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3K9me3

ChIP assays were performed on human HeLa cells using the Diagenode monclonal antibody against H3K9me3 (Cat. No. C15200146). ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), using sheared chromatin from 1,000,000 cells. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. QPCR was performed with primers for the promoters of the active c-fos 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).

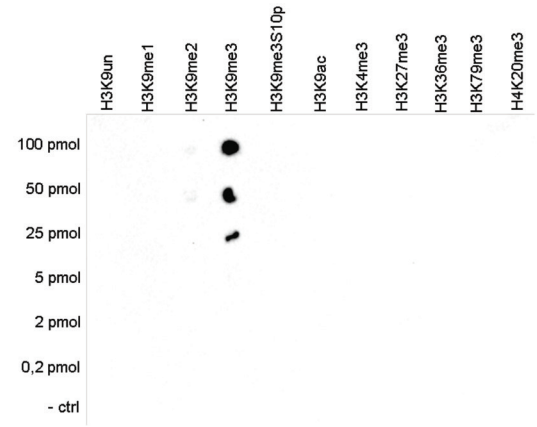

Figure 2. Cross reactivity test using the Diagenode monoclonal antibody directed against H3K9me3

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 2 shows a high specificity of the antibody for the modification of interest.

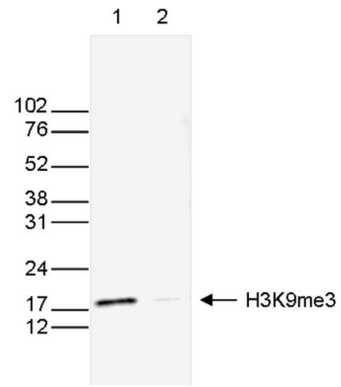

Figure 3. Western blot analysis using the Diagenode monoclonal antibody directed against H3K9me3

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 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.

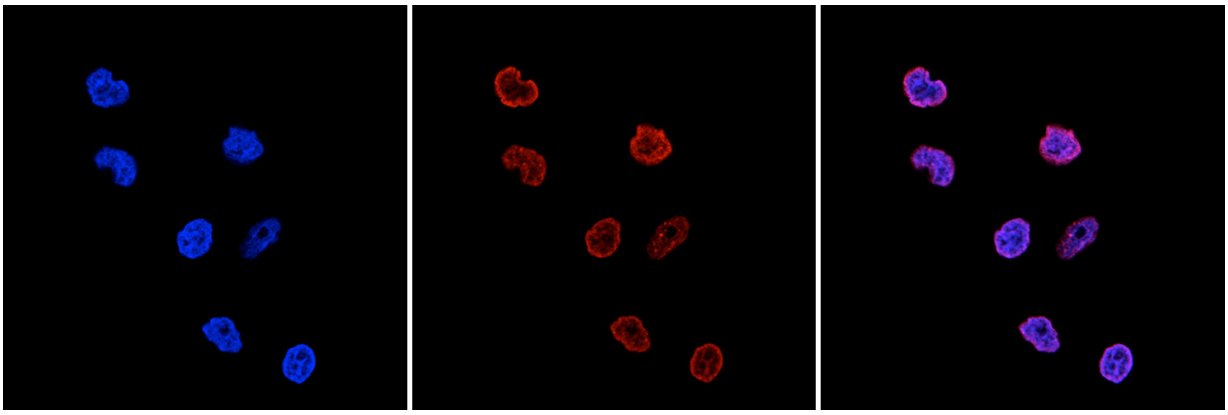

Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K9me3

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.

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