

H3K9me3 monoclonal antibody

Cat. No. C15200153

Type: Monoclonal ChIP-grade

Isotype: IgG1

Source: Mouse

Lot #: 001

Size: 50 µg/ 50 µl

Concentration: 1 µg/µl

Specificity: Human, 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 [1].

Applications

	Suggested dilution	Results
ChIP*	1-3 µg/ChIP	Fig 1
ELISA	1:1,000	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.

This antibody has been described in:

[1] Kimura H, Hayashi-Takanaka Y, Goto Y, Takizawa N and Nozaki N (2008) The organization of histone H3 modifications as revealed by a panel of specific monoclonal antibodies. Cell Struct Funct 33: 61-73.

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.

Results

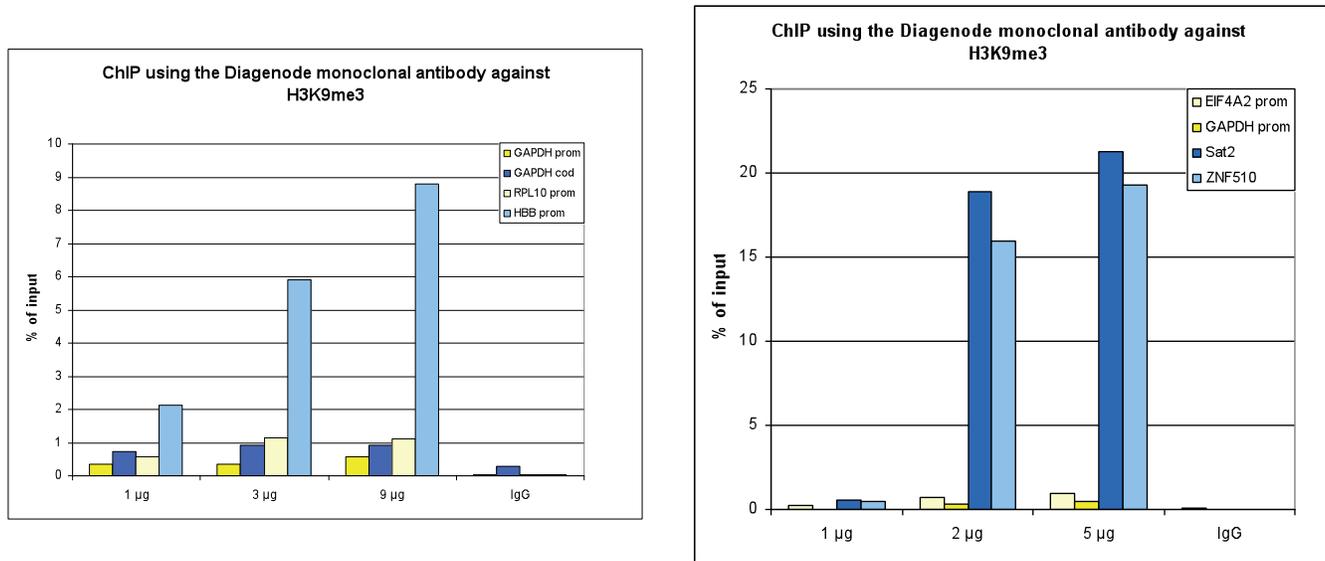


Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3K9me3

A. ChIP was performed using HeLa cells, the monoclonal antibody against H3K9me3 [Cat. No. C15200153] and optimized PCR primer sets for qPCR. Chromatin was sheared with the Diagenode Bioruptor using the “Shearing ChIP” kit [Cat. No. C01020020]. ChIP was performed with the “OneDay ChIP” kit [Cat. No. C01010081], using sheared chromatin from 1.6 million cells. A titration of the antibody consisting of 1, 3 and 9 µg per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoter and the coding region of the GAPDH gene, and for the RPL10 and HBB promoters.

B. ChIP was performed with the “iDeal ChIP” kit [Cat. No. C01010051], using sheared chromatin from 1 million K562 cells. A titration of the antibody consisting of 1, 2 and 5 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoters of the GAPDH and EIF4A2 genes, used as negative controls, and for the ZNF12 gene and the Sat2 satellite repeat, 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).

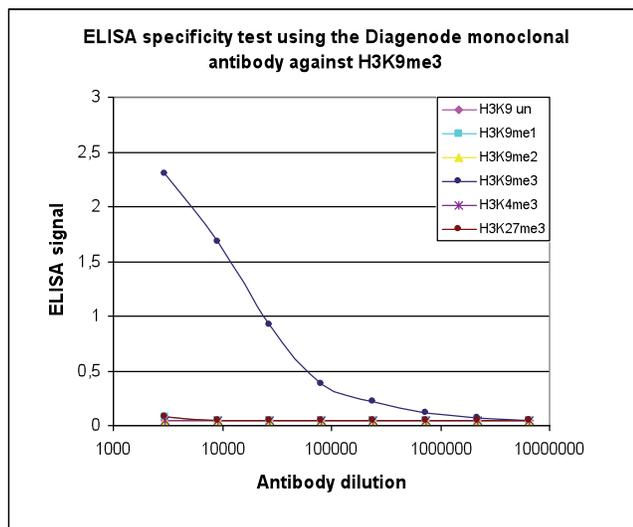


Figure 2. Cross reactivity of the Diagenode monoclonal antibody directed against H3K9me3

To test the specificity an ELISA was performed using a serial dilution of the Diagenode monoclonal antibody against H3K9me3 [Cat. No. C15200153]. The wells were coated with peptides containing the unmodified H3K9 as well as the mono-, di- and trimethylated H3K9 and the trimethylated H3K4 and H3K27. Figure 2 shows a high specificity of the antibody for the modification of interest.

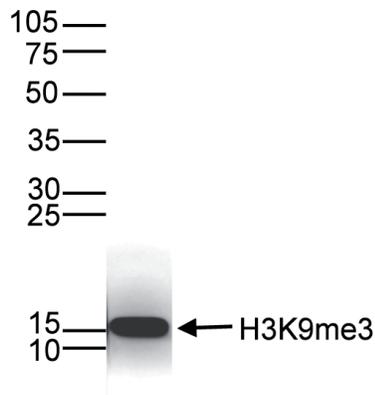


Figure 3. Western blot analysis using the Diagenode monoclonal antibody directed against H3K9me3

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode monoclonal antibody against H3K9me3 (Cat. No. C15200153) 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.

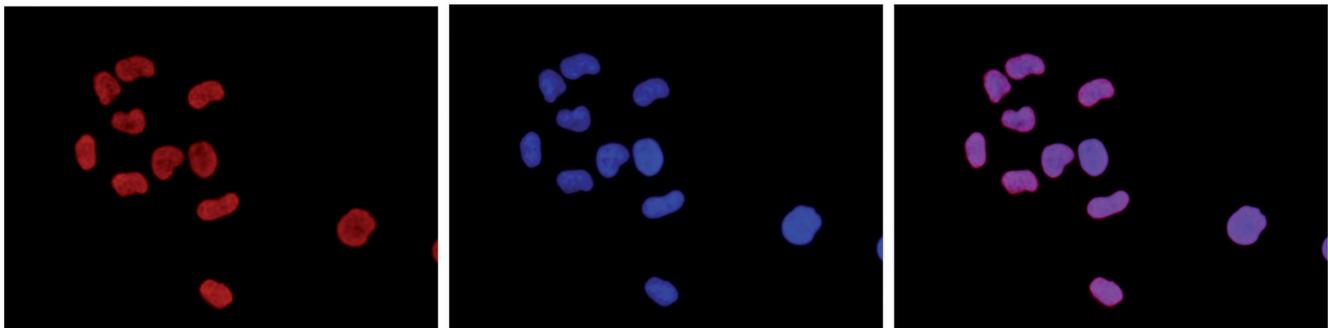


Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K9me3

HeLa cells were stained with the Diagenode antibody against H3K9me3 (Cat. No. C15200153) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K9me3 antibody (left) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.

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