diagenode
Innovating Epigenetic Solutions

TECHNICAL DATASHEET

H3K9me1 polyclonal antibody

Cat. No. C15410065
Type: Polyclonal **ChIP-grade**
Source: Rabbit
Lot #: A89-0041
Size: 50 µg/ 43 µl
Concentration: 1.17 µg/µl

Specificity: Human, Nematodes: positive
Other species: not tested

Purity: Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.

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: Polyclonal antibody raised in rabbit against histone H3 containing the monomethylated lysine 9 (H3K9me1), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP	1 µg per ChIP	Fig 1
ELISA	1:500 - 1:1,000	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:1,000	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 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. Methylation of histone H3K9 is associated with gene repression..

Results

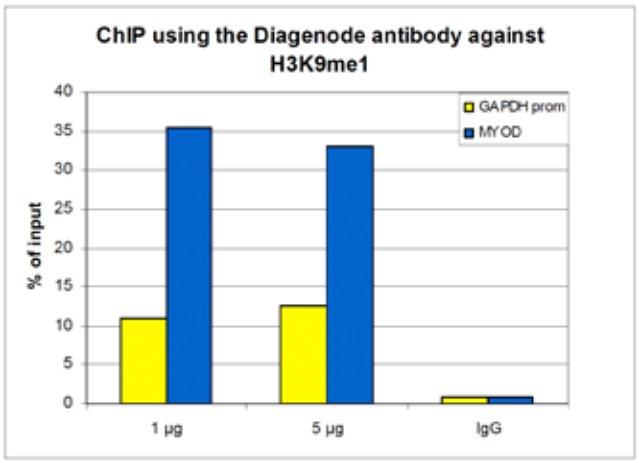

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K9me1

ChIP assays were performed using human HeLa cells, the Diagenode antibody directed against H3K9me1 (Cat. No. C15410065) and optimized PCR primer sets for qPCR. ChIP was performed with the "LowCell# ChIP" kit (Cat. No. C01010072), using sheared chromatin from 10,000 cells. Respectively 1 and 5 µg of the antibody and 5 µg of IgG (negative IP control) were used per ChIP experiment. QPCR was performed with primers for the GAPDH promoter and for the inactive gene MYOD. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis). These results are in accordance with the observation that H3K9me1 is preferably present at silent genes.

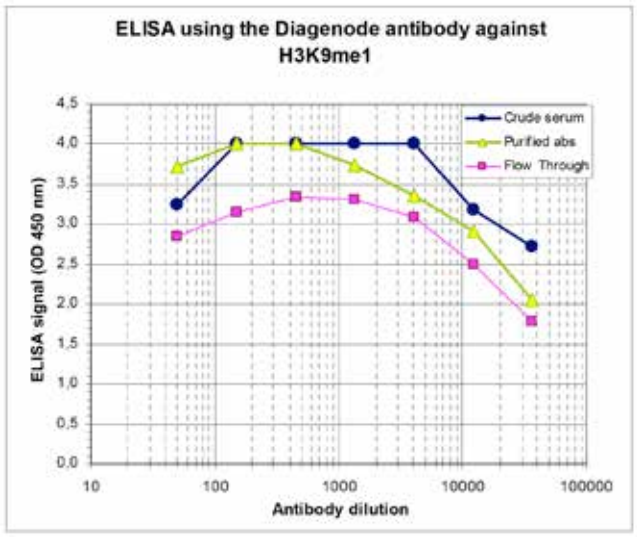

Figure 2. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against human H3K9me1 (Cat. No. C15410065), crude serum and flow through 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 2), the titer of the antibody was estimated to be 1:68,000.

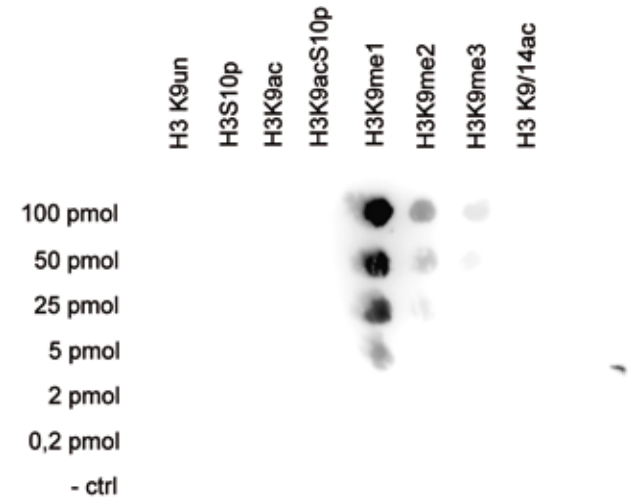

Figure 3. Cross reactivity test of the Diagenode antibody directed against H3K9me1

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9me1 (cat. No. C15410065) with peptides containing other modifications 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 1:20,000. Figure 3 shows a high specificity of the antibody for the modification of interest

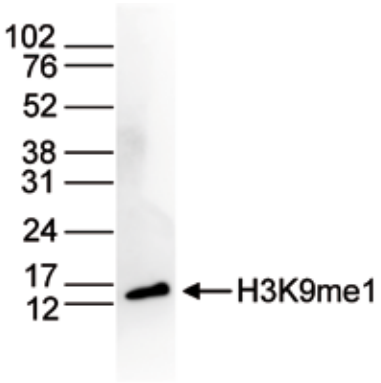

Figure 4. Western blot analysis using the Diagenode antibody directed against H3K9me1

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode antibody against H3K9me1 (Cat. No. C15410065) 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.

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