

PRODUCT NAME H3K9me1 polyclonal antibody		
Cat. No. C15310065 (CS-065-100)	Type: Polyclonal ChIP-grade	Size: 100 µl
Lot #: A89-001	Source: Rabbit	Concentration: not determined

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

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP*	10 µl/ChIP	Fig 1
ELISA	1:2,000 – 1:3,000	Fig 2
Dot blotting	1:200,000	Fig 3
Western blotting	1:1000	Fig 4
Immunofluorescence	1:200	Fig 5

*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µl per IP.

Purity: Whole antiserum from rabbit 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.

Last data sheet update: April 22, 2011

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 and heterochromatin formation, although higher levels of H3K9me1 have been found in some more active promoters.

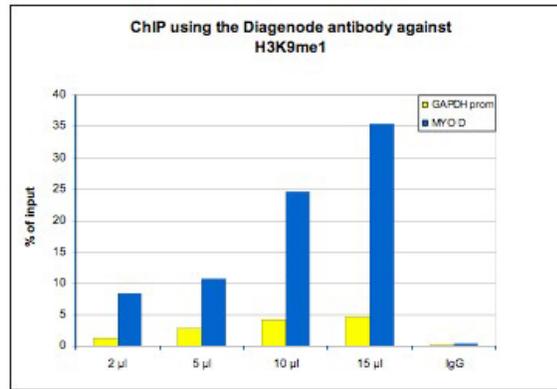


Figure 1

ChIP results obtained with the Diagenode antibody directed against H3K9me1

ChIP assays were performed using human osteosarcoma (U2OS) cells, the Diagenode antibody against H3K9me1 (cat. No. CS-065-100) and optimized PCR primer sets for qPCR. Chromatin was sheared with the Diagenode "Shearing ChIP" kit (cat. No. kch-redmod-100). ChIP was performed with the "OneDay ChIP" kit (cat. No. kch-oneDIP-060), using sheared chromatin from 1.6 million cells. A titration of the antibody consisting of 2, 5, 10 and 15 µl per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control. Quantitative PCR was performed using primers for the promoter of the housekeeping gene GAPDH and for the coding region of the myogenic differentiation gene (MYOD), a gene that is inactive at normal conditions. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

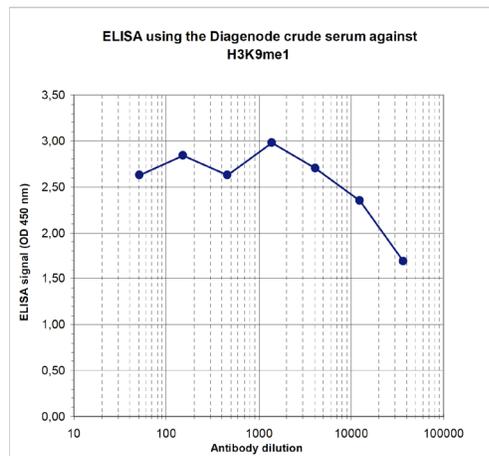


Figure 2

Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K9me1 (cat. No. CS-065-100). 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 1:50,000.

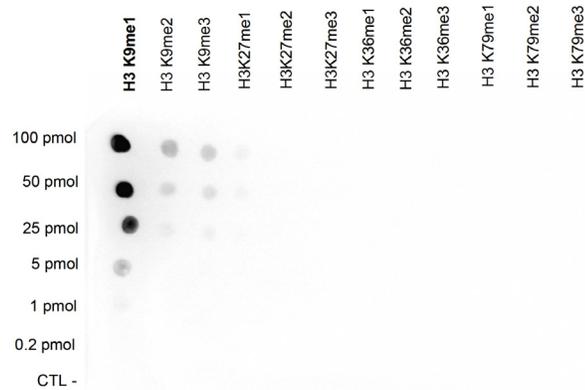


Figure 3

Cross reactivity test using 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. CS-065-100) with peptides containing other modifications of histone H3. Other histone modifications include di- and trimethylation of the same lysine and mono-, di- and trimethylation of lysine 27, 36 and 79. 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 1:200,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

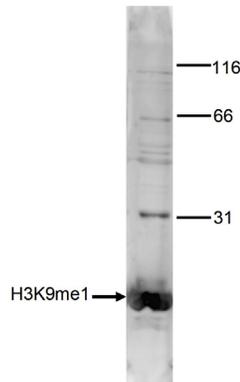


Figure 4

Western blot analysis using the Diagenode antibody directed against H3K9me1

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K9me1 (cat. No. CS-065-100) diluted 1:1000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the left; the marker (in kDa) is shown on the right.

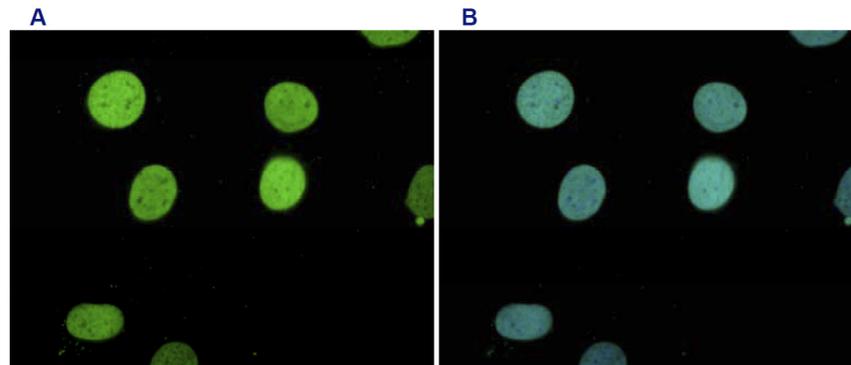


Figure 5

Immunofluorescence using the Diagenode antibody directed against H3K9me1

HeLa cells were stained with the Diagenode antibody against H3K9me1 (cat. No. CS-065-100) and with DAPI. Cells were formaldehyde fixated, permeabilized with Triton X-100 and blocked with PBS containing 2.5% BSA.

Figure 5A: cells were immunofluorescently labelled with the H3K9me1 antibody (diluted 1:200 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to DyLight.

Figure 5B: staining of the nuclei with DAPI, which specifically labels DNA.

Both antibody and DAPI staining are restricted to the nucleus.