TECHNICAL DATA SHEET

PRODUCT NAME H3K9me3 polyclonal antibody		
Cat. No. pAb-056-050	**Type:** Polyclonal **ChIP grade**	**Size:** 50 µg/ 45 µl
Lot #: A93-0042	**Source:** Rabbit	**Concentration:** 1.13 µg/µl

Description: Polyclonal antibody raised in rabbit against the region of histone H3 containing the trimethylated lysine 9 (H3K9me3), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP	2 - 5 µg per ChIP	Fig 1
ELISA	1:500	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:1,000	Fig 4

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.

References citing this antibody:

(1) Dahl J.A. and Collas P. 2008. A rapid micro chromatin immunoprecipitation assay (lChIP). Nature Protocols 3: 1032-45.
(2) Dahl J.A., Reiner A.H. and Collas P. 2009. Fast genomic µChIP-chip from 1,000 cells. Genome Biology 10: R13.

Last data sheet update: August 16, 2010

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. Trimethylation of histone H3K9 is associated with inactive regions.

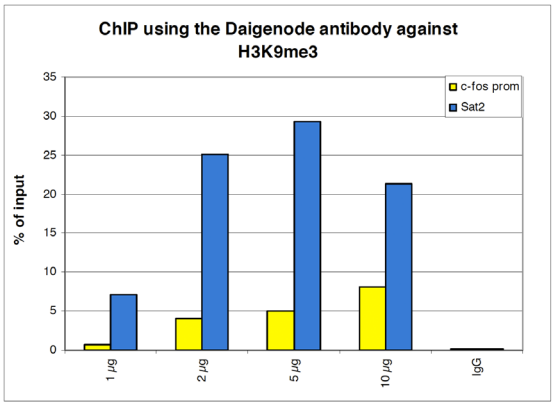

Figure 1

ChIP results obtained with the Diagenode antibody directed against H3K9me3

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K9me3 (cat# pAb-056-050) and optimized PCR primer sets for qPCR. ChIP was performed with the "LowCell# ChIP" kit (cat# kch-maglow-016) on sheared chromatin from 10,000 cells using the SX-8G IP-Star automated system. A titration of the antibody consisting of 1, 2, 5, and 10 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the c-fos promoter (cat# pp-1004-050) and for the heterochromatin marker Sat2 (cat# pp-1040-050). Figure 4 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

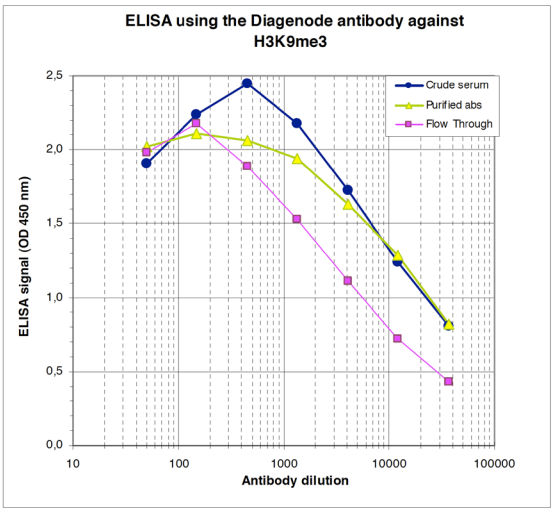

Figure 2

Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody directed against human H3K9me3 (cat# pAb-056-050), 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 1), the titer of the antibody was estimated to be 1:20,500.

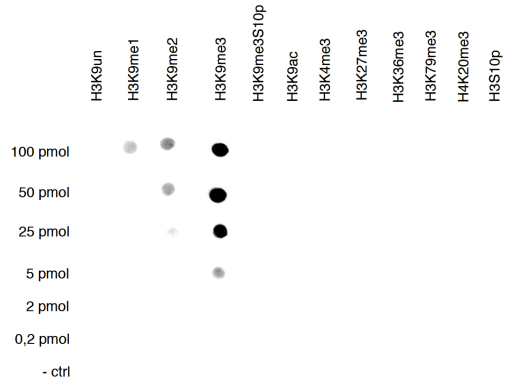

Figure 3

Cross reactivity tests using the Diagenode antibody directed against H3K9me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9me3 (cat# pAb-056-050) with peptides containing other modifications and unmodified sequences 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 2 shows a high specificity of the antibody for the modification of interest.

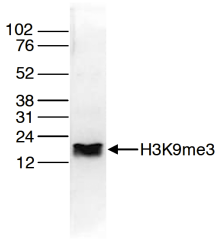

Figure 4

Western blot analysis using the Diagenode antibody directed against H3K9me3

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode antibody against H3K9me3 (cat# pAb-056-050) 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.