TECHNICAL DATA SHEET

PRODUCT NAME H3K27me2 polyclonal antibody		
Cat. No. pAb-046-050	**Type:** Polyclonal **ChIP-grade**	**Size:** 50 µg/ 68 µl
Lot #: A118-00241	**Source:** Rabbit	**Concentration:** 0.73 µg/µl

Description: This antibody has been raised against the region of the histone H3 containing the dimethylated lysine 27 (H3K27me2), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ELISA	1:100 - 1:500	Fig 1
Dot blotting	1:20,000	Fig 2
Western blotting	1:1,000	Fig 3
ChIP	5 µg/ChIP	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.

Last data sheet update: March 1, 2010

References citing this antibody:
(1) Chaturvedi CP, Hosey AM, Palii C, Perez-Iratxeta C, Nakatani Y, Ranish JA, Dilworth FJ, and Brand M (2009) Dual role for the methyltransferase G9a in the maintenance of beta-globin gene transcription in adult erythroid cells. PNAS 106: 18303-18308.

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. Levels of H3K27 dimethylation are higher in silent genes than in active genes suggesting that this histone modification is associated with transcriptional repression.

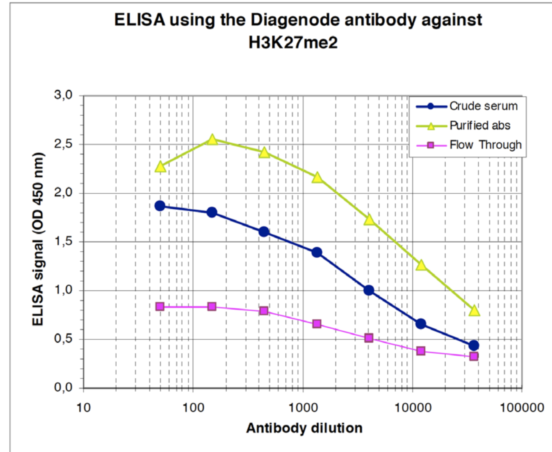

Figure 1

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 H3K27me2 (cat# pAb-046-050), crude serum and flow through. 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 purified antibody was estimated to be 1:16,900.

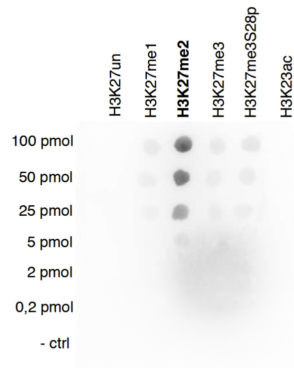

Figure 2

Cross reactivity tests using the Diagenode purified antibody directed against H3K27me2

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K27me2 (cat# pAb-046-050) with peptides containing other modifications and unmodified sequences of histone H3. 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 1:20,000. Figure 2 shows a high specificity of the antibody for the modification of interest.

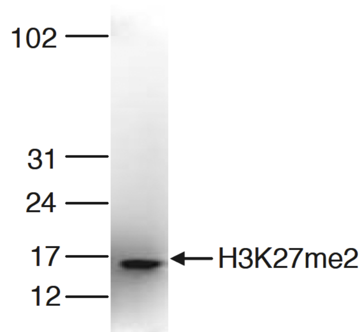

Figure 3

Western blot analysis using the Diagenode antibody directed against H3K27me2

Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the Diagenode antibody against H3K27me2 (cat# pAb-046-050) 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.

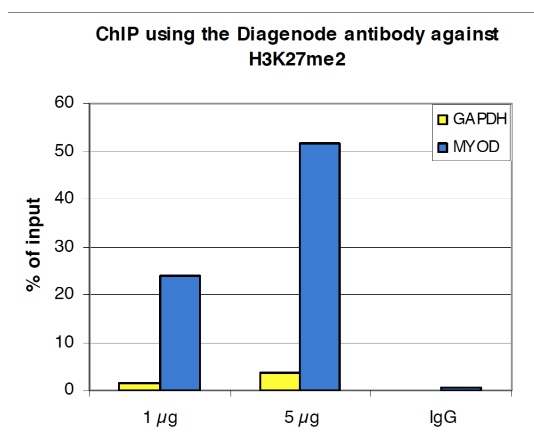

Figure 4

ChIP results obtained with the Diagenode antibody directed against H3K27me2

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K27me2 (cat# pAb-046-050) and optimized PCR primer sets for qPCR. ChIP was performed with the "LowCell# ChIP" kit (cat# kch-maglow-016), using sheared chromatin from 10,000 cells. Two different amounts of antibody (1 and 5 µg per ChIP experiment) were analysed. IgG (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoter of the active gene GAPDH (cat# pp-1001-050) and for the coding region of the myogenic differentiation gene (MYOD), a gene that is inactive at normal conditions. Figure 4 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 H3K27me2 is preferably present at silent genes.