diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME H3K27me3 polyclonal antibody		
Cat. No. C15310069 (CS-069-100)	**Type:** Polyclonal **ChIP-grade**	**Size:** 100 µl
Lot #: A299-001	**Source:** Rabbit	**Concentration:** not determined

Description: Polyclonal antibody raised in rabbit against histone H3 containing the trimethylated lysine 27 (H3K27me3), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP	5-10 µl/ ChIP	Fig 1
ELISA	1:500	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:1,000	Fig 4
Immunofluorescence	1:200	Fig 5

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: February 23, 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.

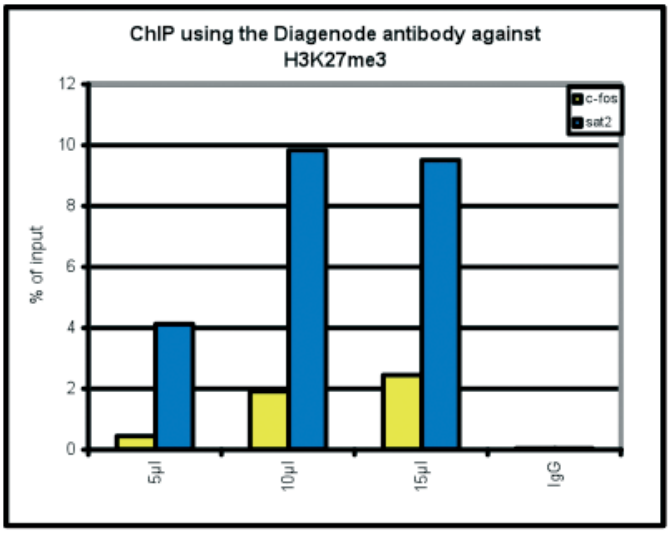

Figure 1

ChIP results obtained with the Diagenode antibody directed against H3K27me3

ChIP assays were performed using HeLa cells, the Diagenode antibody against H3K27me3 (Cat. No. CS-069-100) and optimized primer sets for qPCR. ChIP was performed with the "OneDay ChIP" kit (Cat. No. kch-oneDIP-060), using sheared chromatin from 1.6 million cells. A titration consisting of 5, 10, and 15 µl of antibody per ChIP experiment was analyzed. IgG (5 µg/IP) was used as a negative IP control. PCR was performed with primers specific for the promoter of the constitutively expressed c-fos gene (Cat. No. pp-1004-050, used as a negative control target) and for the satellite repeat Sat2 (Cat. No. pp-1040-050, used as a positive control target). Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

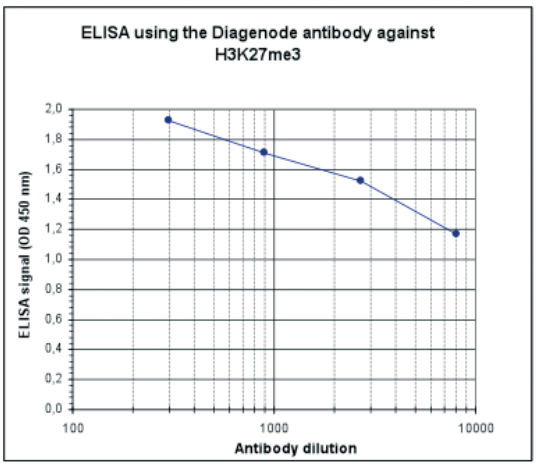

Figure 2

Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Diagenode antibody directed against H3K27me3 (Cat. No. CS-069-100) 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 1:24,700.

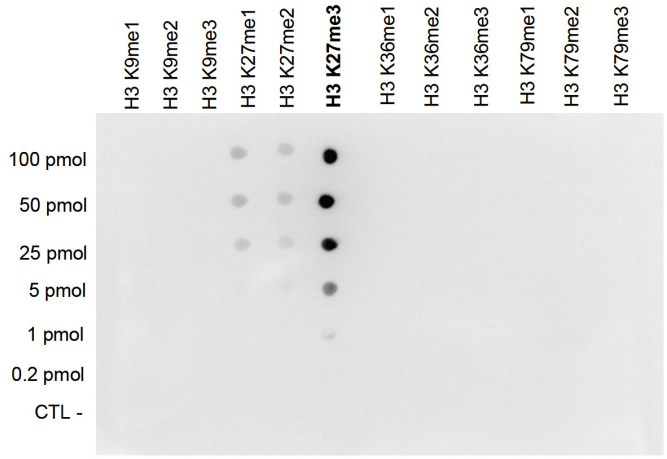

Figure 3

Cross reactivity tests using the Diagenode antibody directed against H3K27me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K27me3 (Cat. No. CS-069-100) with peptides containing other histone modifications including mono- and dimethylation of the same lysine and mono-, di- and trimethylation of other lysines of histone H3. One hundred to 0.2 pmol of the respective peptides 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.

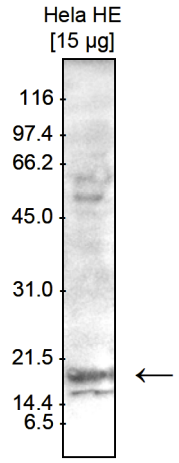

Figure 4

Western blot analysis using the Diagenode antibody against H3K27me3

Histone extracts of HeLa cells (HeLa HE, 15 µg) were analysed by Western blot using the Diagenode antibody against H3K27me3 (Cat. No. CS-069-100) 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.

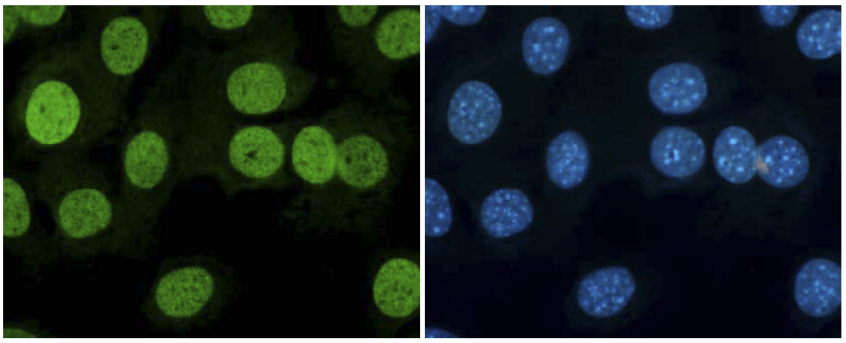

Figure 5

Immunofluorescence using the Diagenode antibody directed against H3K27me3

NIH3T3 mouse fibroblasts were stained with the Diagenode antibody against H3K27me3 (Cat. No. CS-069-100) and with DAPI. Cells were formaldehyde fixed, permeabilized with Triton X100 and then blocked with PBS containing 1% BSA (Figure 5). (A) Cells were immunofluorescently labelled with the H3K27me3 antibody (diluted 1:200 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to FITC. (B) Staining of the nuclei with DAPI, which specifically labels DNA. Both antibody and DAPI staining are restricted to the nucleus. H3K27me3 shows a characteristic broadly dispersed pattern in interphase chromatin.