TECHNICAL DATA SHEET

PRODUCT NAME H3K27me3S28p polyclonal antibody		
Cat. No. C15310091 (CS-091-100)	**Type:** Polyclonal **ChIP-grade**	**Size:** 100 µl
Lot #: A155-001	**Source:** Rabbit	**Concentration:** not determined

Product description: Polyclonal antibody raised in rabbit against histone H3 containing the trimethylated lysine 27 and the phosphorylated serine 28 (H3K27me3S28p), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP*	1 µl/ChIP	Fig 1
ELISA	1:100 – 1:500	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:250 - 1:500	Fig 4, Fig 6
Immunofluorescence	1:200	Fig 5
Immunoprecipitation	5 µl/IP	Fig 6

*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. Phosphorylation of H3 on serine 28 is increased during mitosis.

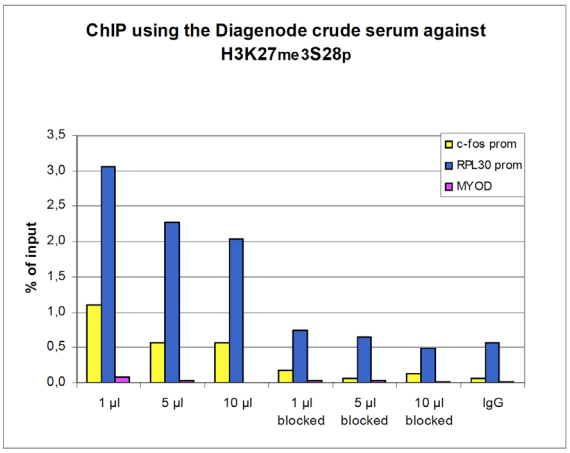

Figure 1

ChIP results obtained with the Diagenode antibody directed against H3K27me3S28p

ChIP assays were performed using HeLa cells treated with colcemid, the Diagenode antibody against H3K27me3S28p (cat. No. CS-091-100) and optimized PCR primer pairs for qPCR. ChIP was performed with the "LowCell# ChIP" kit (cat. No. kch-maglow-016), using sheared chromatin from 10,000 cells. A titration of the antibody consisting of 1, 5 and 10 µl per ChIP experiment was analysed. Additionally, ChIP was performed after incubation of the antibody with 5 nmol blocking peptide (cat. No. sp-091-050) for 1 hour at room temperature. IgG (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoters of the active genes c-fos (cat. No. pp-1004-050) and RPL30 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).

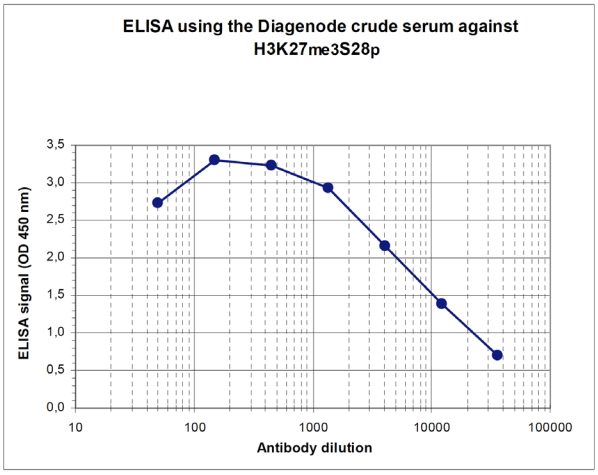

Figure 2

Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K27me3S28p (cat. No. CS-091-100). The antigen used was a peptide containing the histone modifications of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:8,300.

TECHNICAL DATA SHEET

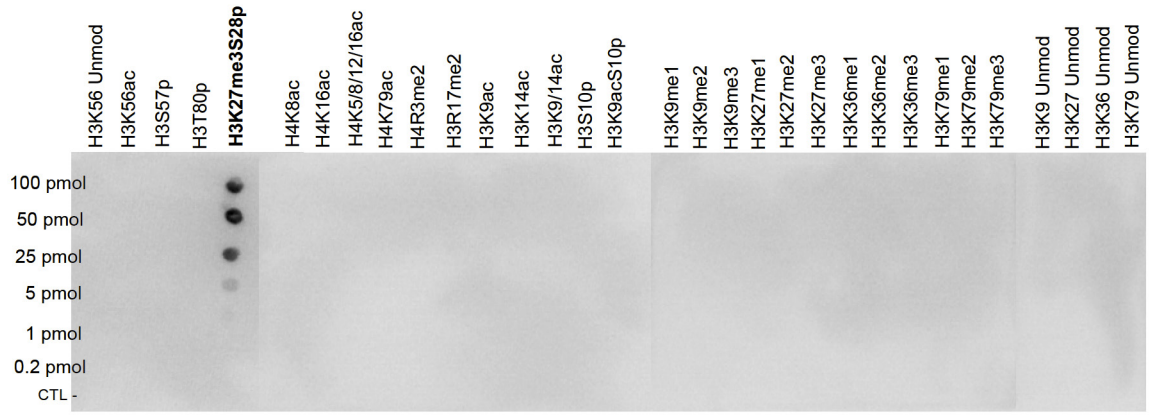

Figure 3

Cross reactivity test using the Diagenode antibody directed against H3K27me3S28p

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K27me3S28p (cat. No. CS-091-100) with peptides containing other modifications of histone H3 and H4 and unmodified sequences from histone H3. One hundred to 0.2 pmol of the 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 peptide containing the modifications of interest.

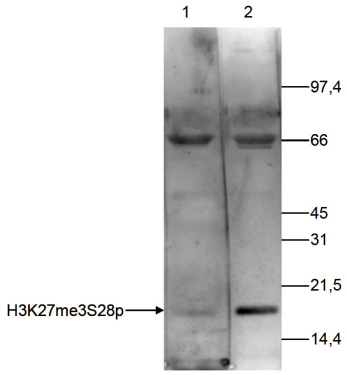

Figure 4

Western blot analysis using the Diagenode antibody directed against H3K27me3S28p

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K27me3S28p (cat. No. CS-091-100) diluted 1:250 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. Lane 2 shows the result of the Western analysis with the antibody; lane 1 shows the same analysis after incubation of the antibody with 750 pmol blocking peptide (cat. No. sp-091-050) for 1 hour at room temperature.

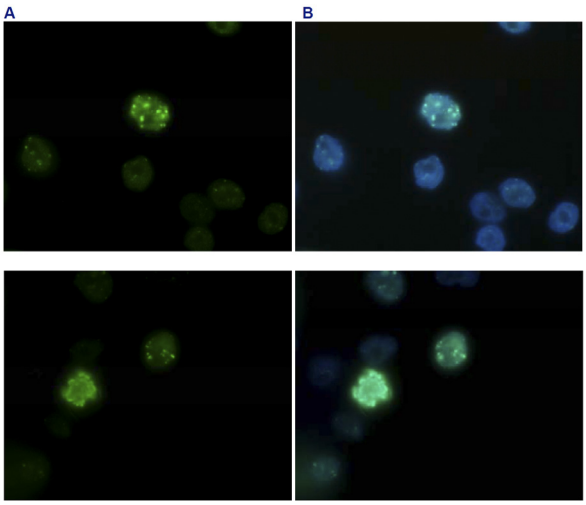

Figure 5

Immunofluorescence with the Diagenode antibody directed against H3K27me3S28p

Hela asynchronous cells were stained with the Diagenode antibody against H3K27me3S28p (cat. No. CS-091-100) and with DAPI. Cells were fixed with formaldehyde, permeabilized with sodium citrate and Triton X100 and blocked with PBS containing 2.5% BSA. Figure 5A: cells were immunofluorescently labelled with the H3K27me3S28p antibody (diluted 1:200 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to DyLight 488. Figure 5B: the nuclei were stained with DAPI, which specifically labels DNA.

Phosphorylation of H3 on serine 28 is increased during late G2 phase and reaches a maximum in metaphase cells. This may explain the different staining intensities of different cells.

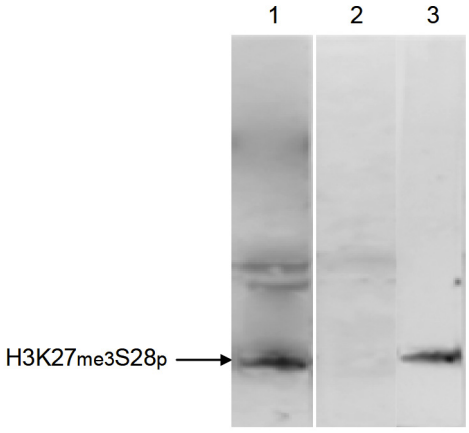

Figure 6

Immunoprecipitation with the Diagenode antibody directed against H3K27me3S28p

HeLa cells were treated with colcemid to block the cell cycle in metaphase and were fixed with formaldehyde. Chromatin from 10,000 cells was sheared and used for immunoprecipitation (IP). IP was performed with 5 µl of the Diagenode antibody against H3K27me3S28p (cat. No. CS-091-100). The immunoprecipitated proteins were analysed by Western blot with the antibody diluted 1:500 in TBS-Tween containing 5% skimmed milk. Lane 1 shows the result of the IP; a negative IP control (no antibody added) and a positive control (sheared chromatin from 10,000 cells) are shown in lane 2 and 3, respectively.