diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME H3S10p polyclonal antibody		
Cat. No. C15410116 (pAb-116-050)	**Type:** Polyclonal **ChIP-grade**	**Size:** 50 µg/ 48 µl
Lot #: A160-001234P	**Source:** Rabbit	**Concentration:** 1.05 µg/µl

Product description: Polyclonal antibody raised in rabbit against histone H3 containing the phosphorylated serine 10 (H3S10p), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP*	2 µg/ChIP	Fig 1
ELISA	1:100	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:1,000	Fig 4
IF	1:2,000	Fig 5

*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

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: April 29, 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 H3S10 is associated with mitosis.

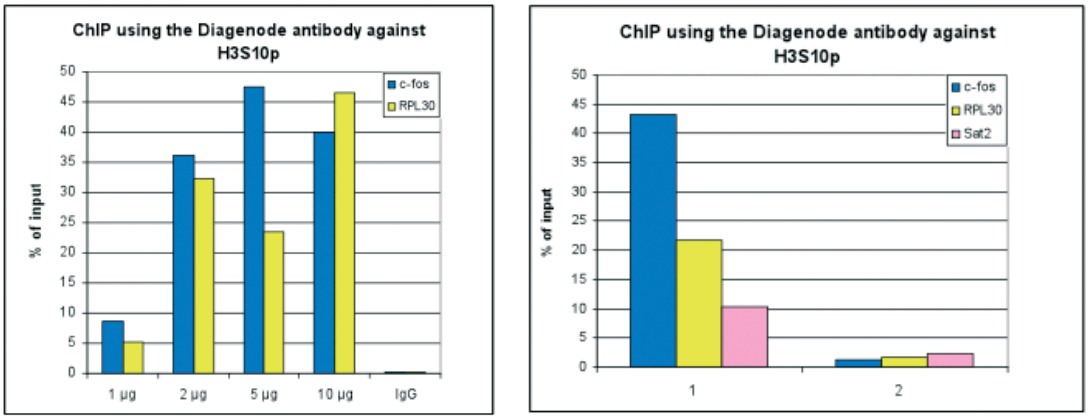


Figure 1
ChIP results obtained with the Diagenode antibody directed against H3S10p

Figure 1A: ChIP assays were performed using human HeLa cells treated with colcemid to block the cells in metaphase, the Diagenode antibody against H3S10p (Cat. No. pAb-116-050) and optimized PCR primer sets for qPCR. ChIP was performed with the "LowCell# ChIP" kit (Cat. No. 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 (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoter of the active genes c-fos (Cat. No. pp-1004-050) and RPL30. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

Figure 1B: ChIP was performed as described above using 2 µg of H3S10p antibody and sheared chromatin from 10,000 HeLa cells treated with colcemid (sample 1) or from 10,000 untreated cells (sample 2). QPCR was performed with primers for the promoter of the active genes c-fos and RPL30, and for the Sat2 satellite repeat region.

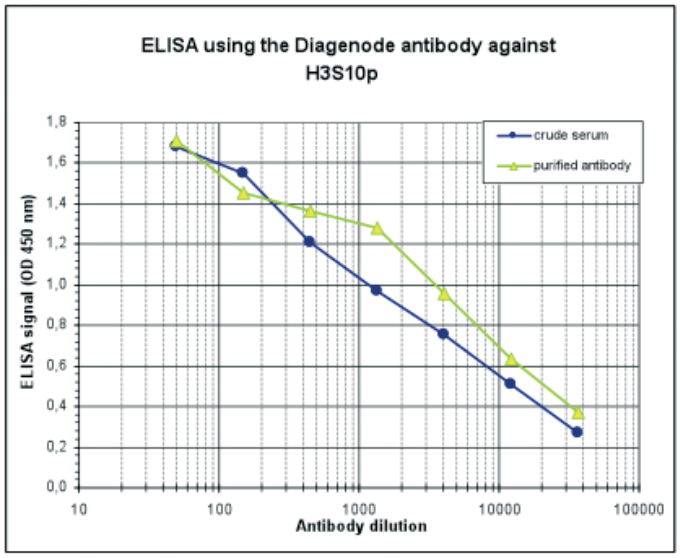


Figure 2

Determination of the antibody titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3S10p (Cat. No. pAb-116-050) and the crude serum. 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 1:5,200.

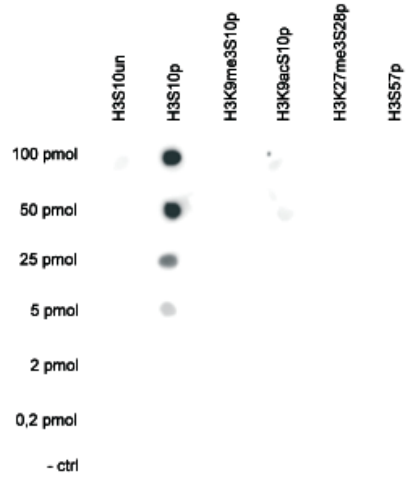


Figure 3

Cross reactivity test with the Diagenode antibody directed against H3S10p

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3S10p (Cat. No. pAb-116-050) with peptides containing other modifications of histone H3 or the unmodified H3S10 sequence. 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 3 shows a high specificity of the antibody for the modification of interest. Note that the antibody does not recognize the H3S10p modification if the neighboring K9 is acetylated or trimethylated.

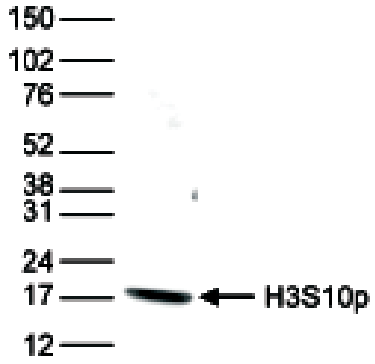
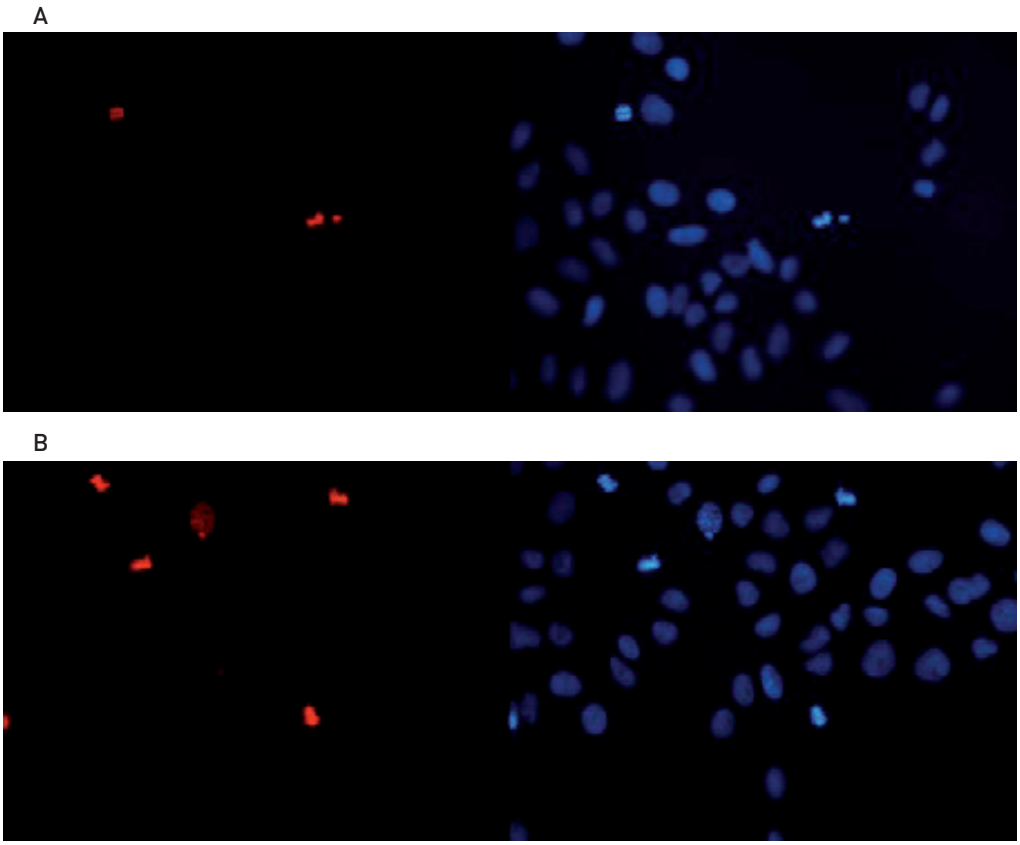


Figure 4

Western blot analysis using the Diagenode antibody directed against H3S10p

HeLa cells were treated with colcemid, which blocks the cell cycle in metaphase and 15 µg of histone extracts of the cells were analysed by Western blot using the Diagenode antibody against H3S10p (Cat. No. pAb-116-050) 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.



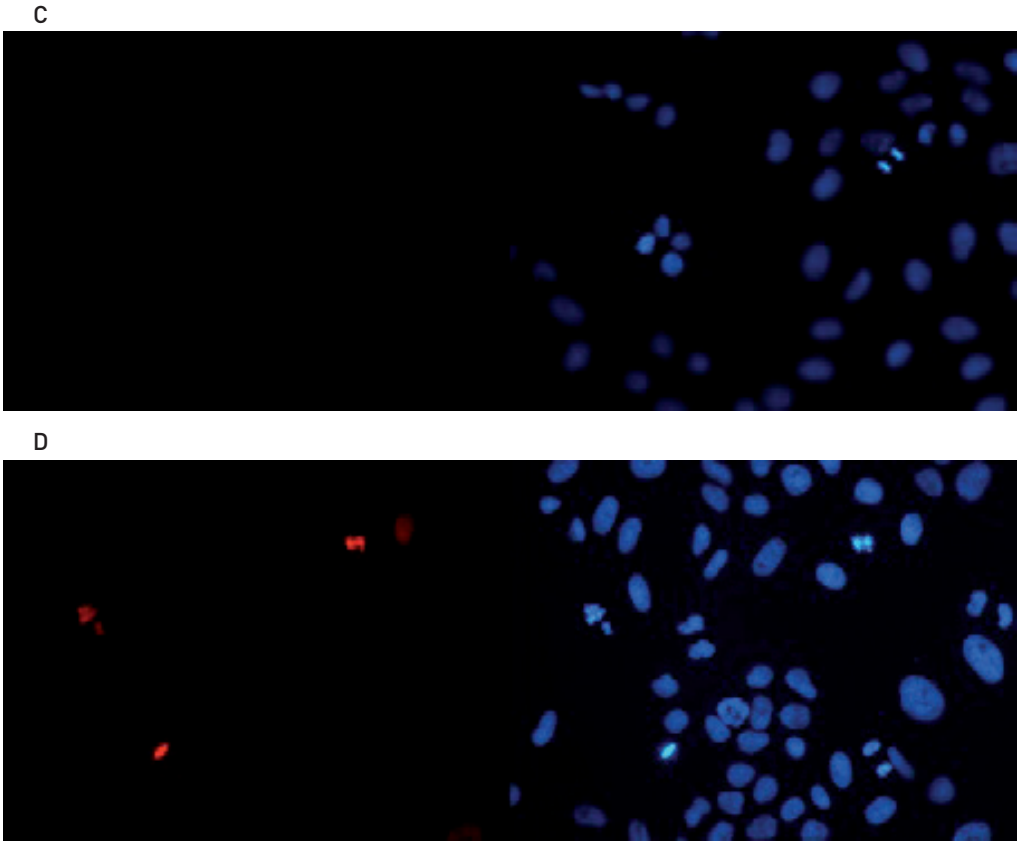


Figure 5
Immunofluorescence using the Diagenode antibody directed against H3S10p
Human osteosarcoma (U2OS) cells were stained with the Diagenode antibody against H3S10p (Cat. No. pAb-116-050) and with DAPI. Cells were fixed with 3.7% formaldehyde in PBS for 20' at RT, followed by a 20' permeabilization with 0.5% Triton X-100 in PBS and blocked PBS/TX-100 containing 5% normal goat serum

Figure 5A: cells were immunofluorescently labeled with the H3S10p antibody (left), diluted 1:2,000 in blocking buffer followed by an anti-rabbit antibody conjugated to Alexa568 or with DAPI (right), which specifically labels DNA.

Figure 5B, C and D: staining of the cells with the H3S10p antibody after incubation of the antibody with 2 µM blocking peptide containing the unmodified H3S10 sequence, the phosphorylated H3S10 and the phosphorylated H3T11, respectively.