

| PRODUCT NAME<br>H3S10p polyclonal antibody |                                           |                                      |
|--------------------------------------------|-------------------------------------------|--------------------------------------|
| <b>Catalog #:</b> C15310116 (CS-116-100)   | <b>Type:</b> Polyclonal <b>ChIP-grade</b> | <b>Size:</b> 100 µl                  |
| <b>Lot #:</b> A161-001                     | <b>Source:</b> Rabbit                     | <b>Concentration:</b> not determined |

**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*               | 1 µl/ChIP          | Fig 1        |
| ELISA               | 1:1,000 – 1:5,000  | Fig 2        |
| Dot blotting        | 1:20,000           | Fig 3        |
| Western blotting    | 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.

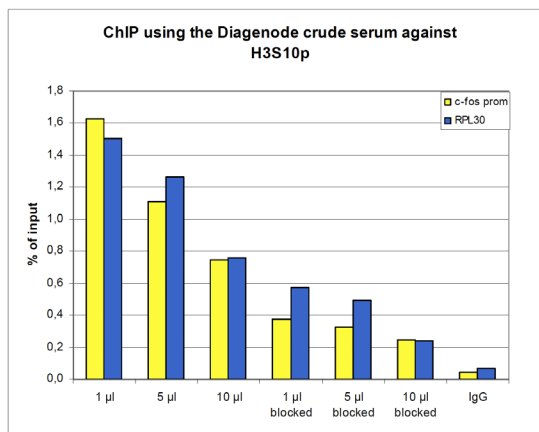
**Storage:** Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

**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**

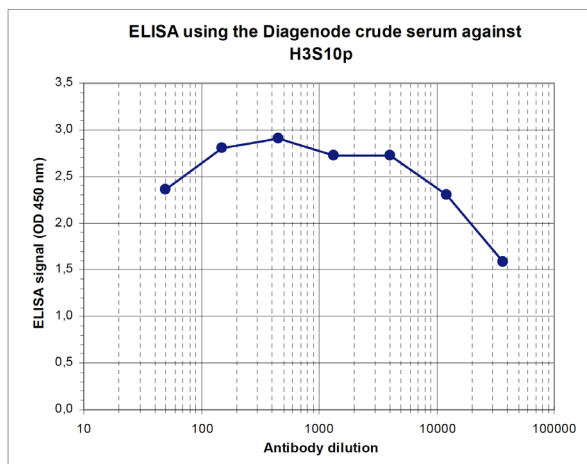
Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble 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 H3S10 is associated with mitosis.



**Figure 1**

**ChIP results obtained with the Diagenode antibody directed against H3S10p**

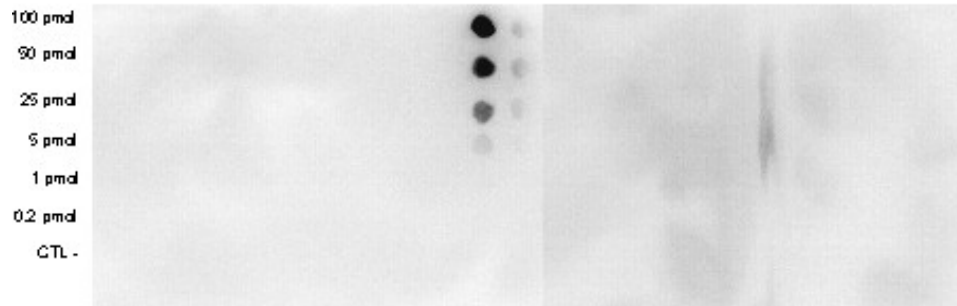
ChIP assays were performed using human HeLa cells treated with colcemid, the Diagenode antibody against H3S10p (cat. No. CS-116-100) and optimized PCR primer sets for qPCR. ChIP was performed with the “LowCell# ChIP” kit (cat. No. kch-maglow-016), using sheared chromatin from 10,000 cells. A titration 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-116-050) for 1 hour at room temperature. 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 2**

**Determination of the titer**

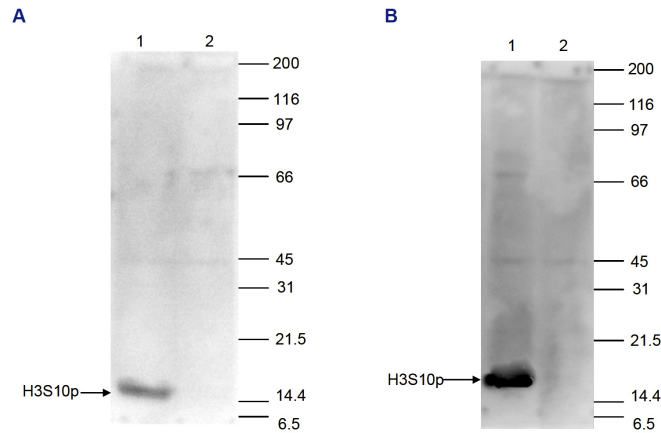
To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against human H3S10p (cat. No. CS-116-100). The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:35,000



**Figure 3**

**Cross reactivity test with the Diagenode antibody directed against H3S10p**

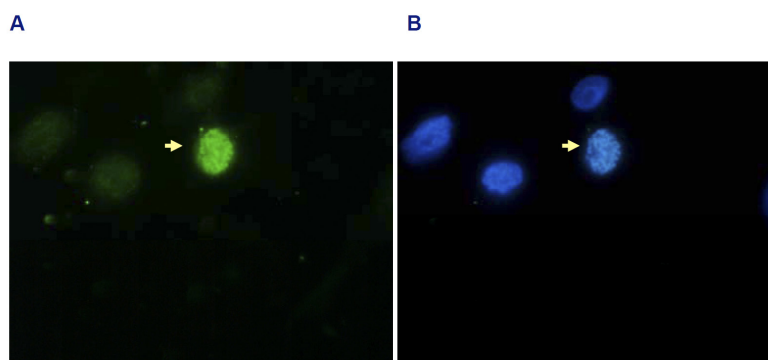
A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3S10p (cat. No. CS-116-100) with peptides containing other modifications of histone H3 and H4 and with peptides containing unmodified sequences from histone H3. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 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 H3K9ac modification is present.



**Figure 4**

**Western blot analysis using the Diagenode antibody directed against H3S10p**

HeLa cells were treated with TSA (figure 4A) or with colcemid (figure 4B), and 15 µg of histone extracts of these cells were analysed by Western blot using the Diagenode antibody against H3S10p (cat. No. CS-116-100) diluted 1:500 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. The result of the Western analysis with the antibody is shown in lane 1; lane 2 shows the same analysis after incubation of the antibody with 750 pmol blocking peptide (cat. No. sp-116-050) for 1 hour at room temperature.

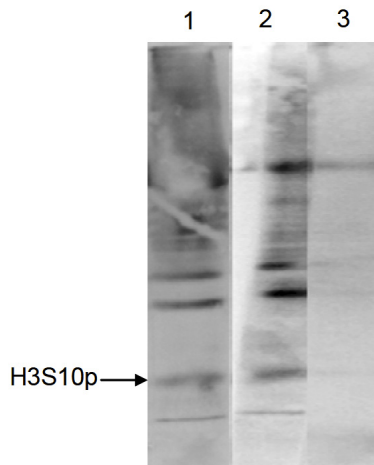


**Figure 5**

**Immunofluorescence with the Diagenode antibody directed against H3S10p**

HeLa asynchronous cells were stained with the Diagenode antibody against H3S10p (cat. No. CS-116-100) and with DAPI. Cells were fixed with formaldehyde, permeabilized with sodium citrate and Triton X100 and blocked with PBS containing 2.5% BSA. (A) Cells were immunofluorescently labelled with the H3S10p antibody (diluted 1:200 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to DyLight 488. (B) The nuclei were stained with DAPI, which specifically labels DNA.

Phosphorylation of H3 on serine 10 occurs on condensed chromosomes during mitosis. This explains the dense staining of one of the cells (indicated with an arrow) in Figure 5.



**Figure 6**

**Immunoprecipitation with the Diagenode antibody directed against H3S10p**

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 H3S10p (cat. No. CS-116-100). 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 positive control (sheared chromatin from 10,000 cells) and a negative IP control (no antibody added) are shown in lane 2 and 3, respectively.