diagenode
A Hologic Company

TECHNICAL DATASHEET

# H3K9me3S10p polyclonal antibody

**Cat. No. C15310128** (CS-128-100)
**Type:** Polyclonal **ChIP grade**
**Source:** Rabbit
**Lot #:** A609-001
**Size:** 100 µl
**Concentration:** Not determined

**Specificity:** Human: positive / Other species: not tested
**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.

**Description:** Polyclonal antibody raised in rabbit against histone H3 containing trimethylated lysine 9 and the phosphorylated serine 10 (H3K9me3S10p), using a KLH-conjugated synthetic peptide.

## Applications

| | Suggested dilution | Results |
|---|---|---|
| ChIP* | 5 µl/ChIP | Fig 1 |
| ELISA | 1:1,000 – 1:10,000 | Fig 2 |
| Dot blotting | 1:1,000 | Fig 3 |
| Western blotting | 1:500 | Fig 4 |
| IF | 1:500 | Fig 5 |

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µg per IP.

## 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.

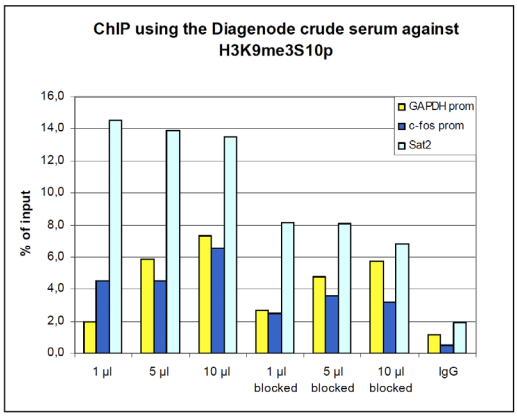


**Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K9me3S10p**

ChIP assays were performed using human HeLa cells treated with colcemid, the Diagenode antibody against H3K9me3S10p (cat. No. CS-128-100) 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 per IP. A titration of the antibody consisting of 1, 5, and 10 µl per ChIP experiment was analysed. Additionally, the same titration was analysed after incubation of the antibody with 5 nmol blocking peptide (cat. No. sp-128-050) 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 GAPDH (cat. No. pp-1001-050) and c-fos (cat. No. pp-1004-050) and for the heterochromatin marker Sat2 (cat. No. pp-1040-050). Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

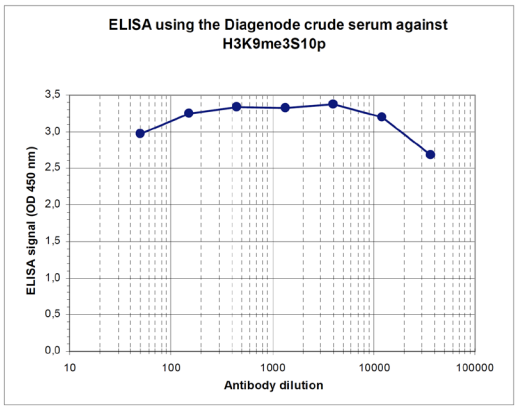


**Figure 2. Determination of the titer**

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against human H3K9me3S10p (cat. No. CS-128-100). 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 1:87,000.

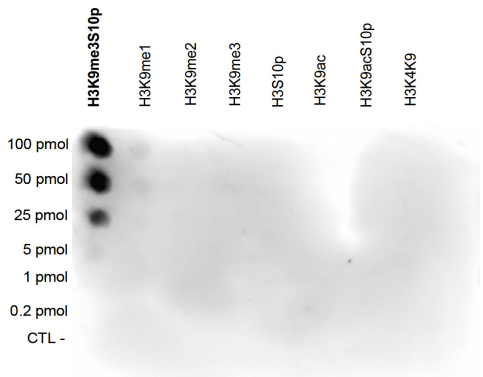


**Figure 3. Cross reactivity test using the Diagenode antibody directed against H3K9me3S10p**

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9me3S10p (cat. No. CS-128-100) with peptides containing other modifications and unmodified sequences of 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 1:1,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

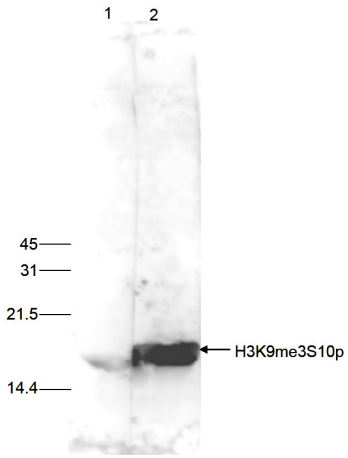


**Figure 4. Western blot analysis using the Diagenode antibody directed against H3K9me3S10p**

HeLa cells were treated with colcemid to block the cell cycle in metaphase and 15 µg of histone extracts of these cells were analysed by Western blot with the Diagenode antibody against H3K9me3S10p (cat. No. CS-128-100) diluted 1:500 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. The result of the Western analysis with the antibody is shown in lane 2; lane 1 shows the same analysis after incubation of the antibody with 5 nmol blocking peptide (cat. No. sp-128-050) for 1 hour at room temperature.

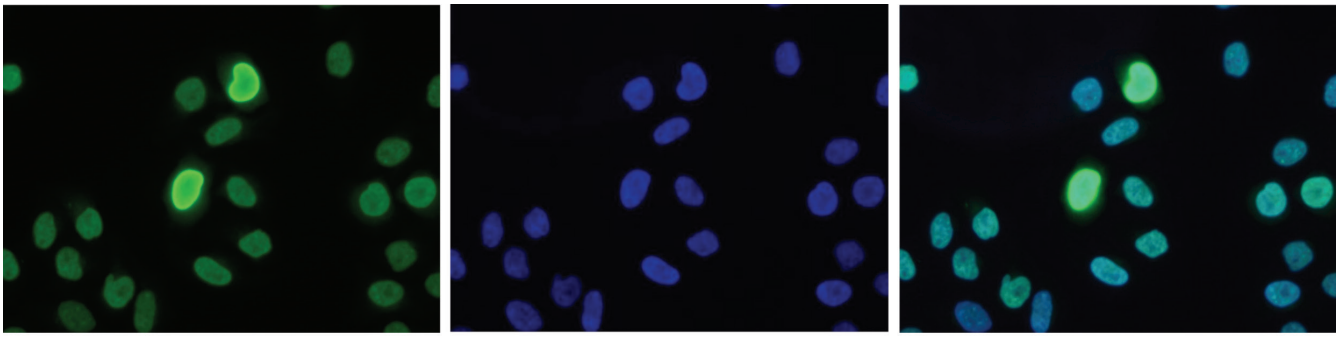

**Figure 5. Immunofluorescence using the Diagenode antibody directed against H3K9me3S10p**

HeLa cells were stained with the Diagenode antibody against H3K9me3S10p (cat. No. CS-128-100) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K9me3S10p antibody (left) diluted 1:500 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.

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