H3K9acS10p Antibody - ChIP Grade

Cat. No. C15310102

Type: Polyclonal	Specificity: Human
Size: 100 µl	Isotype: NA
Concentration: not determined	Host: Rabbit
Lot No.: A397-001	Purity: Whole antiserum
Storage buffer: NA	Storage conditions: NA
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 the **acetylated lysine 9 and the phosphorylated serine 10 (H3K9acS10p)**, using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP *	15 µl/ChIP	Fig 1
ELISA	1:1,000 - 1:4,000	Fig 2
Dot Blotting	1:20,000	Fig 3
Western Blotting	1:250	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 µl 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. Acetylation of K9 and phosphorylation of S10 of histone H3 are associated with active gene transcription.

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Validation Data

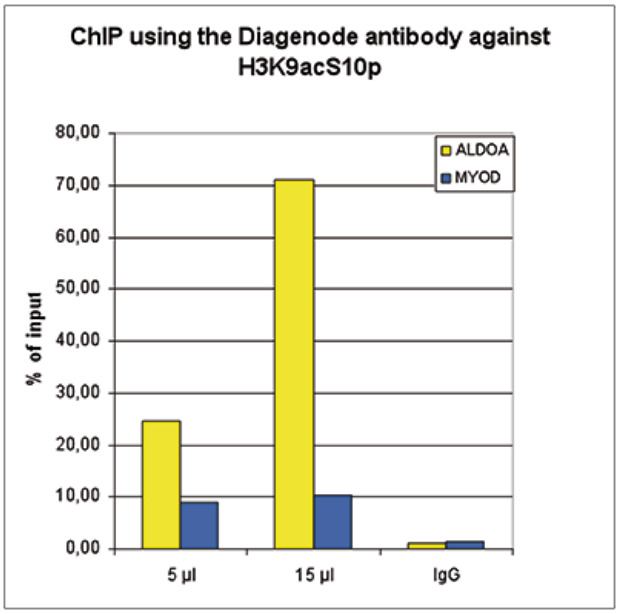

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K9acS10p

ChIP assays were performed using human osteosarcoma (U2OS) cells, the Diagenode antibody against H3K9acS10p (Cat. No. CS-102-100) and optimized PCR primer sets for qPCR. Chromatin was sheared with the Diagenode "Shearing ChIP" kit (Cat. No. kch-redmod-100). ChIP was performed with the "OneDay ChIP" kit (Cat. No. kch-oneDIP-060), using sheared chromatin from 1.5 million cells. A titration of the antibody consisting of 5 and 15 µl per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the ALDOA (fructose-bisphosphate aldolase A) promoter and for the coding region of the myogenic differentiation gene (MYOD), a gene that is inactive at normal conditions. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

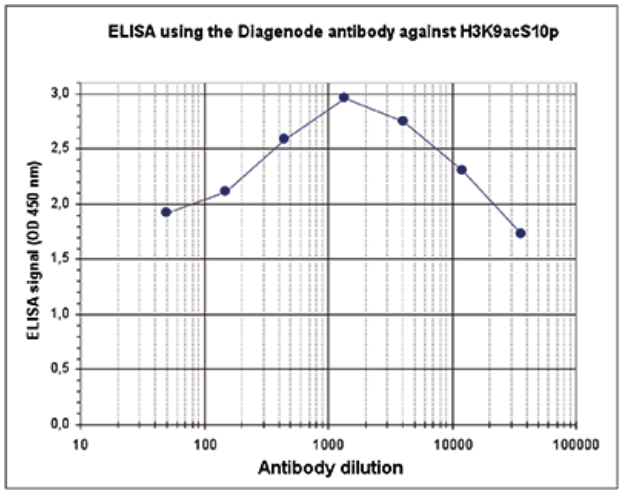

Figure 2. Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody against human H3K9acS10p (Cat. No. CS-102-100). 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 1:89,000.

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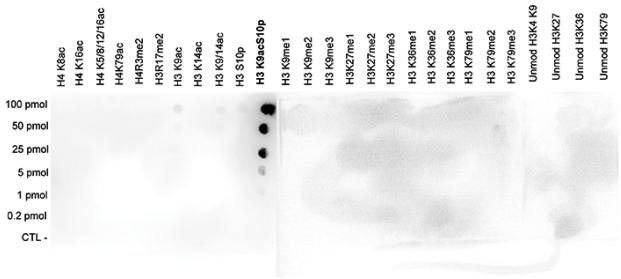

Figure 3. Cross reactivity test using the Diagenode antibody directed against H3K9acS10p

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K9acS10p (Cat. No. CS-102-100) with peptides containing other modifications of histone H4 and H3 or unmodified histone H3 sequences. 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 double modification.

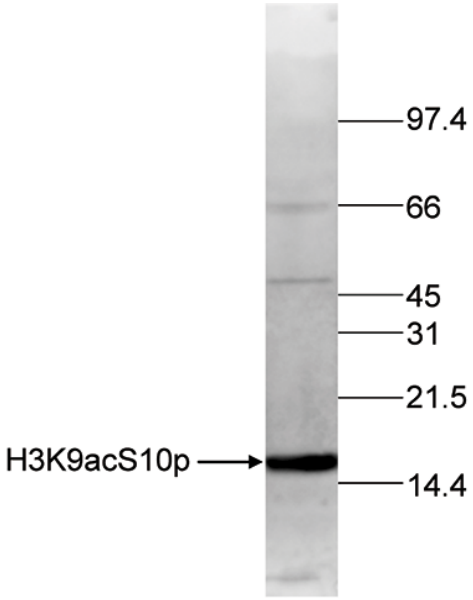

Figure 4. Western blot analysis using the Diagenode antibody directed against H3K9acS10p

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K9acS10p (Cat. No. CS-102-100) 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.

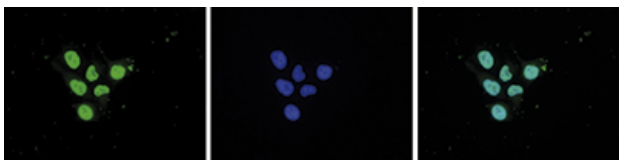

Figure 5. Immunofluorescence using the Diagenode antibody directed against H3K9acS10p

HeLa cells were stained with the Diagenode antibody against H3K9acS10p (cat. No. C15310102) 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 H3K9acS10p 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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