diagenode
Innovating Epigenetic Solutions

H3T11p polyclonal antibody - Classic

Cat. No. C15410309

Type: Polyclonal **ChIP-grade**

Source: Rabbit

Lot #: A2288P

Size: 50 µg/36 µl

Concentration: 1.4 µg/µl

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 the region of histone H3 containing the phosphorylated Threonine 11 (H3T11p), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution/amount	Results
ChIP*	5 µg/ChIP	Fig 1
ELISA	1:1,000	Fig 2
Dot blotting	1:20,000	Fig 3
WB	1:1,000	Fig 4
IF	1:200	Fig 5

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, H2A, 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.

Results

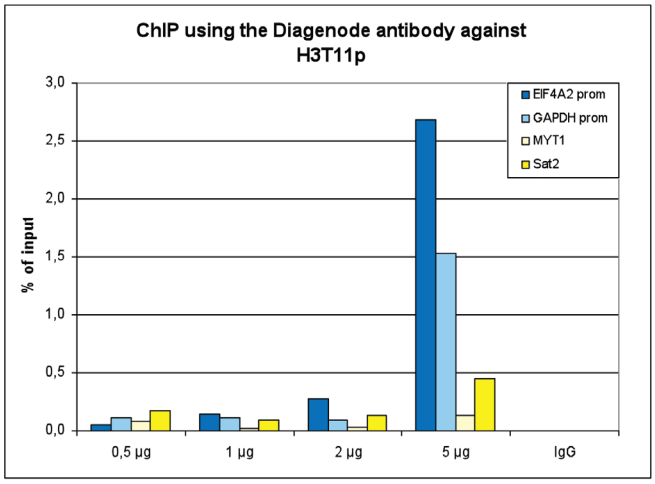
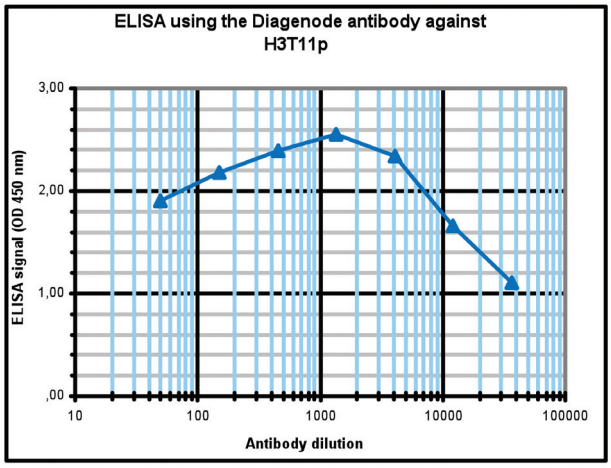


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3T11p

ChIP assays were performed using human HeLa cells, treated with colcemid, the Diagenode antibody against H3T11p (cat. No. C15410309) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (cat. No. C01010055), using sheared chromatin from 1.5 million cells. A titration of the antibody consisting of 0.5, 1, 2 and 5 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QPCR was performed with primers for the EIF4A2 and GAPDH promoters, used as positive controls, and for the coding region of the inactive MYT1 gene and the Sat2 satellite repeat, used as negative controls. 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 antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3T11p (cat. No. C15410309) in antigen coated wells. 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 1:25,700.



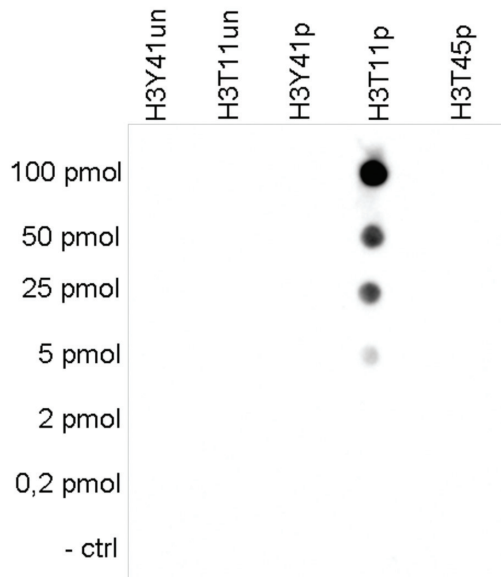
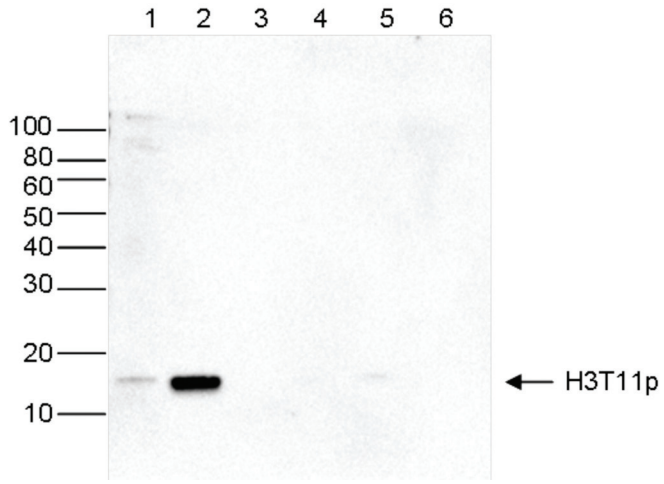


Figure 3. Cross reactivity tests using the Diagenode antibody directed against H3T11p

To test the cross reactivity of the Diagenode antibody against H3T11p (cat. No. C15410309), a Dot Blot analysis was performed with peptides containing other histone phosphorylations and the unmodified H3T11. One hundred to 0.2 pmol of the respective 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 modification of interest.

Figure 4. Western blot analysis using the Diagenode antibody directed against H3T11p

Western blot was performed on whole cell extracts from untreated HeLa cells (25 µg, lane 1), on histone extracts from HeLa cells treated with colcemid (15 µg, lane 2), and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the Diagenode antibody against H3T11p (cat. No. C15410309). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.



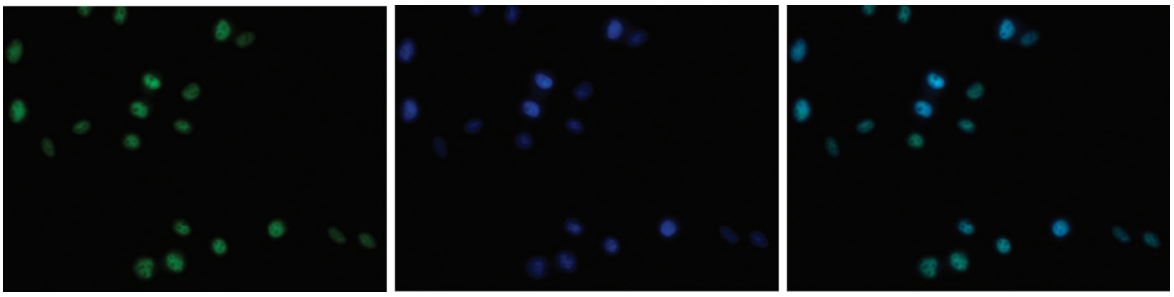


Figure 5. Immunofluorescence using the Diagenode antibody directed against H3T11p

HeLa cells were treated with colcemid and stained with the Diagenode antibody against H3T11p (cat. No. C15410309) 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 H3T11p antibody (left) diluted 1:200 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.

Diagenode sa. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode Inc. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

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