diagenode
A Hologic Company

TECHNICAL DATASHEET

H4pan polyclonal antibody

Cat. No. C15410156
Type: Polyclonal **ChIP-grade**
Source: Rabbit
Lot #: A1217P
Size: 50 µg/ 39 µl
Concentration: 1.3 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 histone H4 using a KLH-conjugated synthetic peptide containing an unmodified sequence from the C-terminal part of the protein.

Applications

	Suggested dilution	Results
ChIP*	1 - 2 µg/ChIP	Fig 1
ELISA	1:100	Fig 2
Western blotting	1:1,000	Fig 3
IF	1:1,000	Fig 4

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 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. Histones play a central role in the regulation of transcription, DNA repair, DNA replication and chromosomal stability. These different functions are established via a complex set of post-translational modifications which either directly or indirectly alter chromatin structure and DNA accessibility to facilitate transcriptional activation or repression or other nuclear processes.

Results

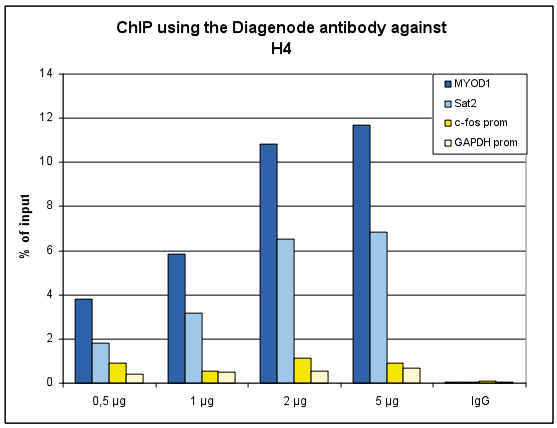

Figure 1. ChIP results obtained with the Diagenode antibody directed against H4

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H4 (Cat. No. C15410156) and optimized PCR primer pairs for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit (Cat. No. C01010022), using sheared chromatin from 1 million cells on the IP-Star Compact automated system. A titration consisting of 0.5, 1, 2 and 5 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoters of the active GAPDH and c-fos genes, and for the inactive MYOD1 gene and the Sat2 satellite repeat. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

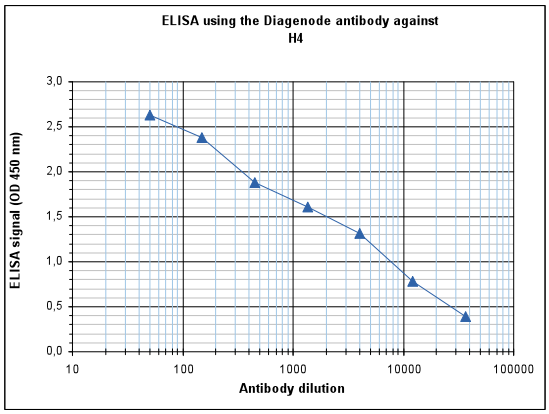

Figure 2. Determination of the titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H4 (Cat. No. C15410156) in antigen coated wells. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:3,000.

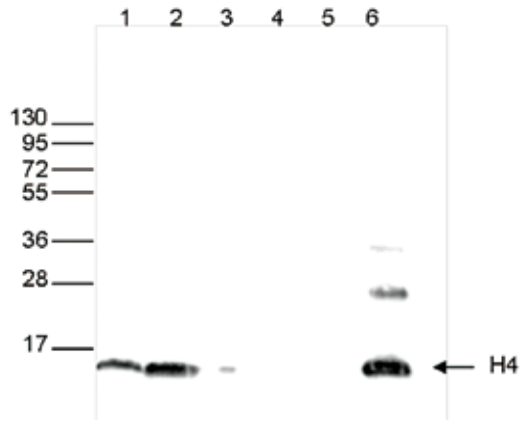

Figure 3. Western blot analysis using the Diagenode antibody directed against H4

Western blot was performed on whole cell extracts (25 µg, lane 1) and histone extracts (15 µg, lane 2) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the Diagenode antibody against H4 (Cat. No. C15410156). The antibody was 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.

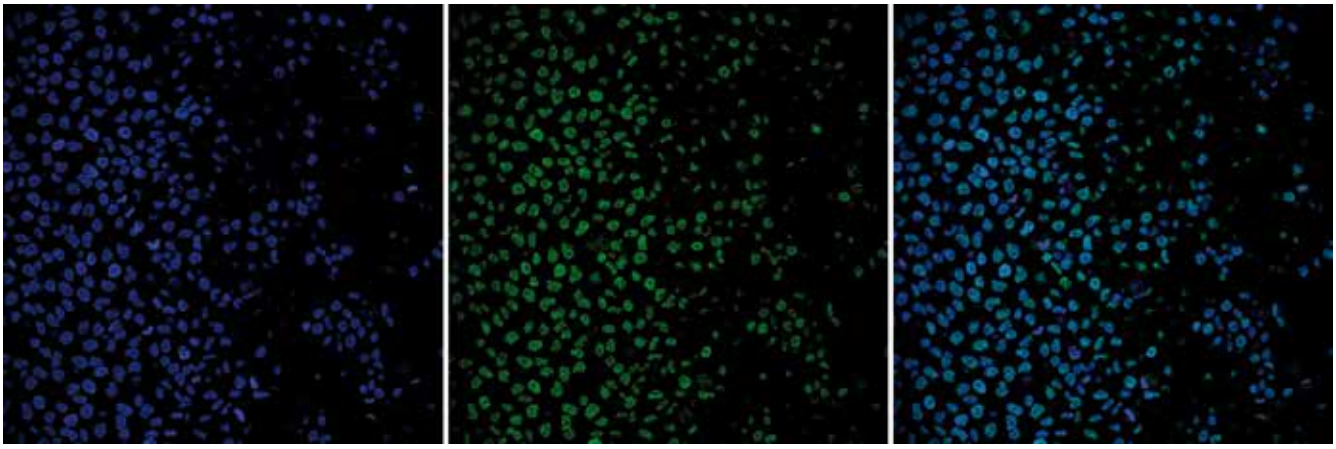

Figure 4. Immunofluorescence using the Diagenode antibody directed against H4

HeLa cells were stained with the Diagenode antibody against H4 (Cat. No. C15410156) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labeled with the H4 antibody (middle) diluted 1:1,000 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The left panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.

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