

H4pan antibody

Cat. No. C15410156

Lot:	A1217P
Size:	10 µg / 50 µg
Type:	Polyclonal, ChIP-grade
Isotype:	NA
Source:	Rabbit
Concentration:	1.3 µg/µl

Specificity:	Human: positive Other species: not tested
Purity:	Affinity purified polyclonal antibody
Storage buffer:	PBS containing 0.05% azide and 0.05% ProClin 300.

Storage: Store at -20 °C. For long-term 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

Applications	Suggested dilution	References
ChIP*	1–2 µg per ChIP	Fig 1
ELISA	1:500	Fig 2
Western blotting	1:1,000	Fig 3
Immunofluorescence	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 the 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 are dynamically regulated by histone methyltransferases and histone demethylases, respectively. Trimethylation of histone H3K9 is associated with inactive regions of chromatin.

Diagenode, SA BELGIUM | EUROPE

LIEGE SCIENCE PARK
Rue du Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders.diagenode@hologic.com
support.diagenode@hologic.com

Diagenode, LLC 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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Results

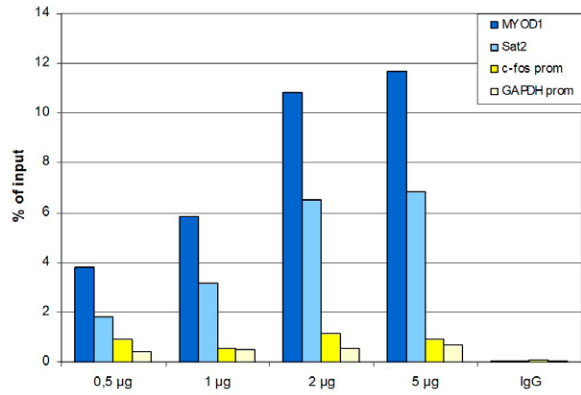


Figure 1: ChIP results obtained with the antibody directed against H4

ChIP assays were performed using human HeLa cells, the antibody against H4 (cat. no. C15410156), and optimized PCR primer pairs for qPCR. ChIP was performed on sheared chromatin from 1 million cells. 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 % input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

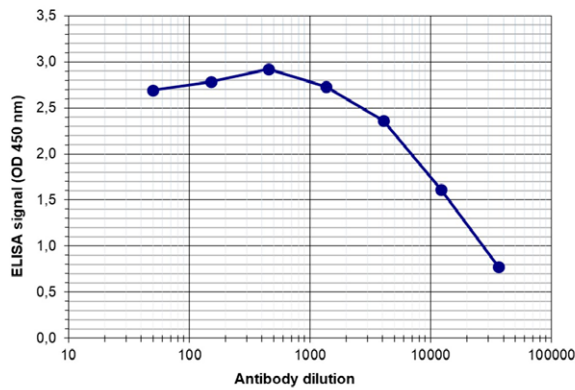


Figure 2: Determination of the titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the 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 1:14,400.

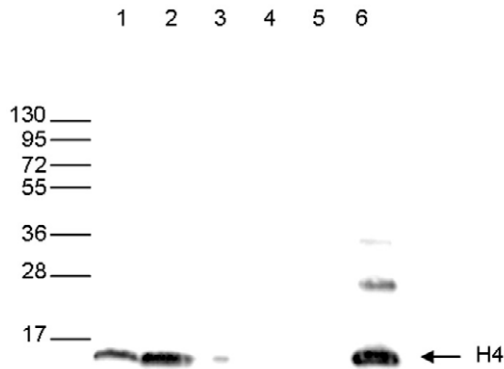


Figure 3: Western blot analysis using the antibody directed against H4

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 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.

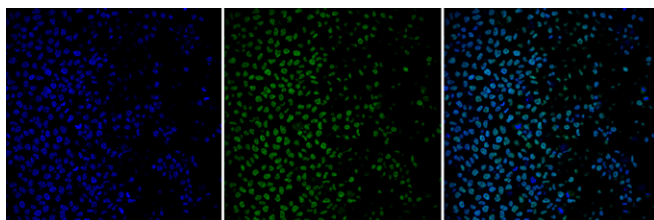


Figure 4: Immunofluorescence using the antibody directed against H4

HeLa cells were stained with the antibody against H4 (cat. no. C15410156) and with DAPI. Cells were fixed with 4% formaldehyde for 10 min 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 stains is shown on the right.