diagenode
A Hologic Company

TECHNICAL DATASHEET

H3pan polyclonal antibody

Cat. No. C15310135

Type: Polyclonal	Specificity: Human, zebrafish, Daphnia: positive. Other species: not tested.
Size: 100 µl	Isotype: NA
Concentration: Not determined	Host: Rabbit
Lot No.: A2566-001	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

This antibody has been raised in rabbit against two KLH-conjugated synthetic peptides containing an unmodified sequence from the central part and from the C-terminus of histone H3, respectively.

Applications

Applications	Suggested dilution	References
ChIP	1 µl/ChIP	Fig 1
ELISA	1:10,000	Fig 2
Western Blotting	1:500	Fig 3

* Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 ?g 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.

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Validation Data

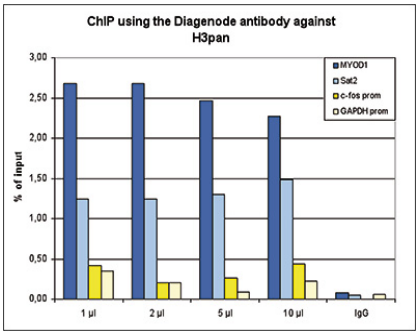

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3pan

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3pan (Cat. No. C15310135) and optimized PCR primer sets for qPCR. ChIP was performed with the Auto Histone ChIP-seq kit (Cat. No. C01010022), using sheared chromatin from 1 million cells. A titration of the antibody consisting of 1, 2, 5, and 10 ?l per ChIP experiment was analysed. IgG (2 ?g/IP) was used as negative IP control. QPCR was performed with primers for the promoters of the active GAPDH and EIF4A2 genes, used as negative controls, and for the inactive MYOD1 and the Sat2 satellite repeat, used as positive controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

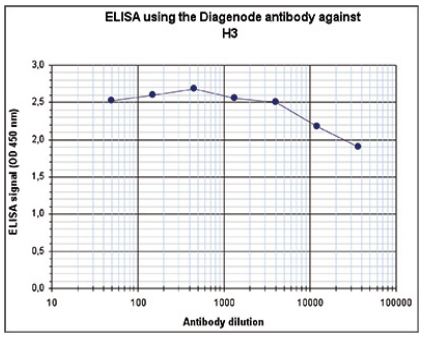

Figure 2. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3pan (Cat. No. C15310135). The plates were coated with the peptides used for immunization. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be >1:1,000,000.

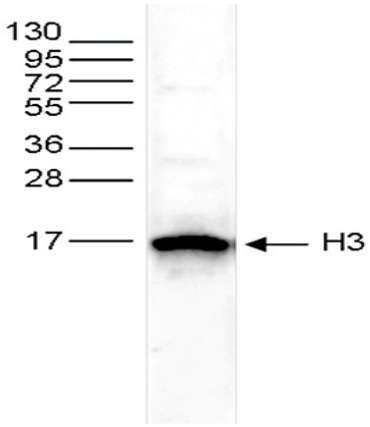

Figure 3. Western blot analysis using the Diagenode antibody directed against H3pan

Whole cell extracts from HeLa cells (25 ?g) were analysed by Western blot using the Diagenode antibody against H3pan (Cat. No. C15310135) 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.

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