H2Bpan polyclonal antibody

Cat. No. C15410157

Type: Polyclonal	Specificity: Human
Size: 50 µg/18 µl	Isotype: NA
Concentration: 2.8 µg/µl	Source: Rabbit
Lot No.: A2622P	Purity: Peptide affinity purified
Storage buffer: PBS containing 0.05% azide.	Storage conditions: 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.	

Last Data Sheet Update: May 3, 2018

Description

Polyclonal antibody raised in rabbit against histone H2B using a KLH-conjugated synthetic peptide containing an unmodified sequence from the C-terminal part of the protein.

Applications

Applications	Suggested dilution	References
ChIP *	0.5 - 1 µg/IP	Fig 1
ELISA	1:1,000 - 1/5,000	Fig 2
WB	1:2,000 - 1/10,000	Fig 3
IF	1:1,000	Fig 3

* 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.

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Validation Data

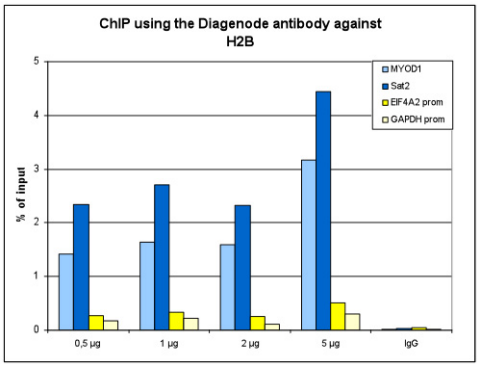

Figure 1 ChIP results obtained with the Diagenode antibody directed against H2B

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H2B (cat. No. C15410157) and optimized PCR primer sets for qPCR. ChIP was performed with the Auto Histone ChIP-seq" kit (Cat. No. C01010022) on sheared chromatin from 1 million cells using the IP-Star automated system. 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 promoters of the active GAPDH and EIF4A2 genes, used as negative controls and for the inactive MYOD1 gene 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.

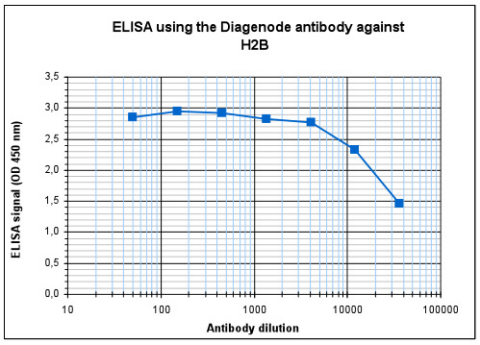

Figure 2 Determination of the titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H2B (C15410157) in antigen coated wells. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:40,000.

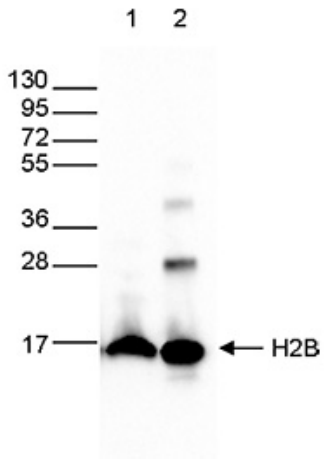

Figure 3 Western blot analysis using the Diagenode antibody directed against H2B

Western blot was performed on whole cell extracts from HeLa cells (40 µg, lane 1) and on 1 µg of recombinant histone H2B (lane 2) using the Diagenode antibody against H2B (Cat. No. C15410157). The antibody was diluted 1:10,000 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.

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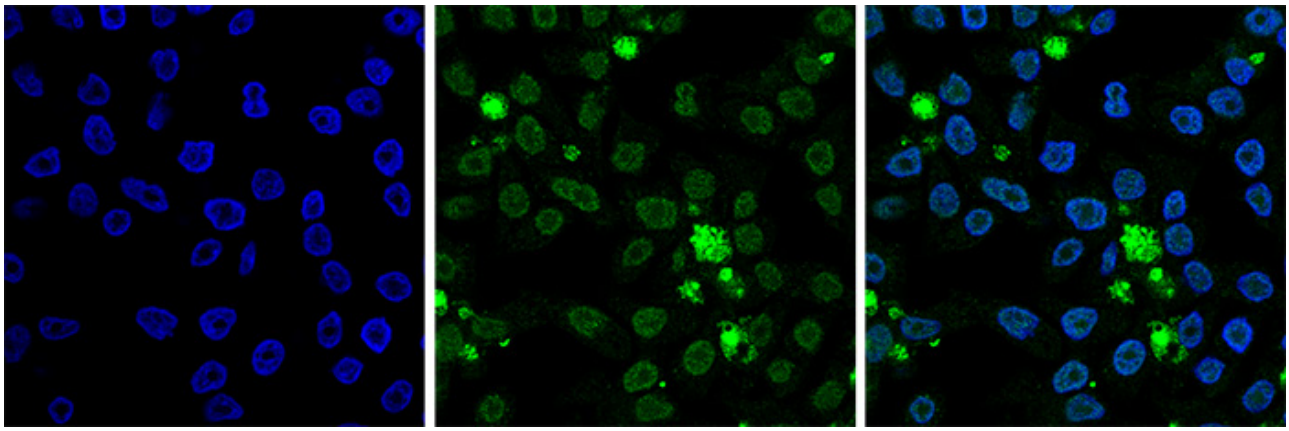

Immunofluorescence using the Diagenode antibody directed against H2B

HeLa cells were stained with the Diagenode antibody against H2B (Cat. No. C15410157) 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 H2B 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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