diagenode
A Hologic Company

TECHNICAL DATASHEET

H2BK15ac polyclonal antibody

Cat. No. C15410220
Type: Polyclonal / ChIP-grade / ChIP-seq grade
Source: Rabbit
Lot #: A2247-0040
Size: 50 µg / 24 µl
Concentration: 2.1 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 H2B containing the acetylated lysine 15 (H2BK15ac), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP *	2 µg per ChIP	Fig 1, 2
ELISA	1:1,000	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:500	Fig 5
IF	1:500	Fig 6

* 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. 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. Acetylation of histone H2B is associated with active genes.

Results

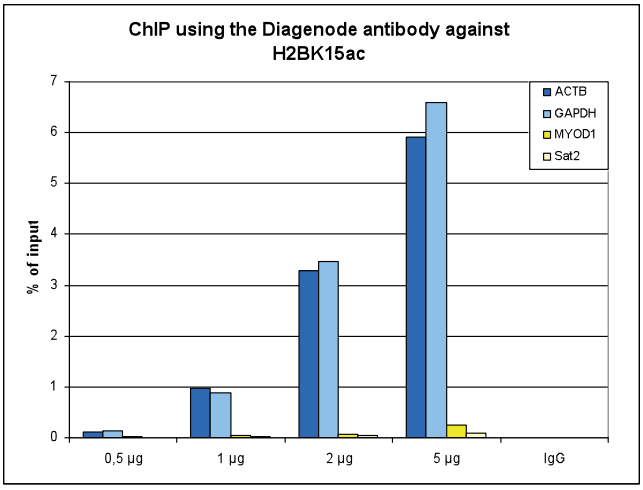


Figure 1. ChIP results obtained with the Diagenode antibody directed against H2BK15ac

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H2BK15ac (Cat. No. C15410220) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), 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 (1 µg/IP) was used as negative IP control. QPCR was performed with primers for a region approximately 1 kb upstream of the GAPDH and ACTB promoters, used as positive controls, and for the coding region of the inactive MYOD1 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).

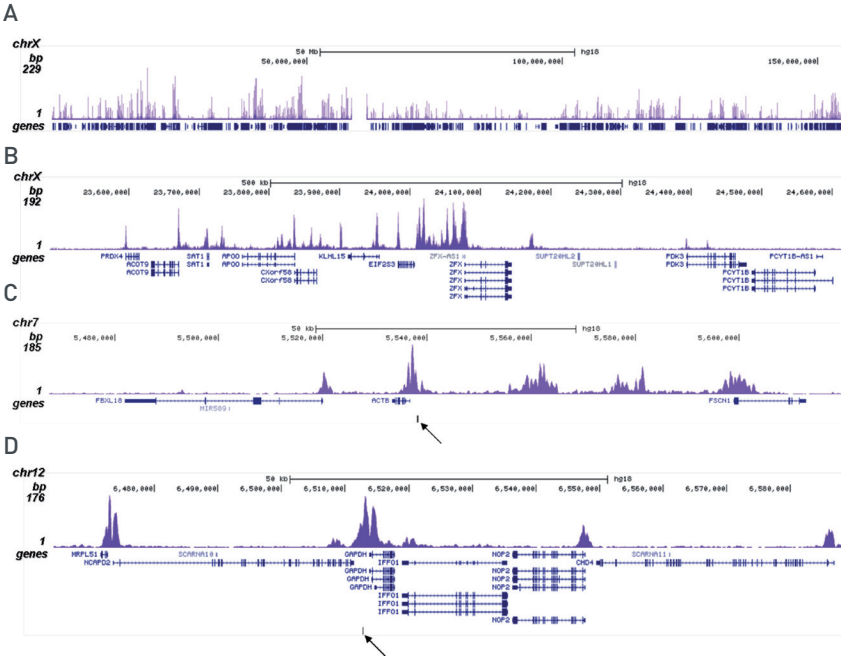


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H2BK15ac

ChIP was performed on sheared chromatin from 1.5 million HeLaS3 cells using 2 µg of the Diagenode antibody against H2BK12ac (Cat. No. C15410220) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment along the complete sequence and a 1 Mb region of the X-chromosome (fig 2A and B) and in genomic regions of chromosome 7, surrounding the ACTB gene, and of chromosome 12, surrounding the GAPDH gene (fig 2C and D). The position of the amplicon used for ChIP-qPCR is indicated by an arrow.

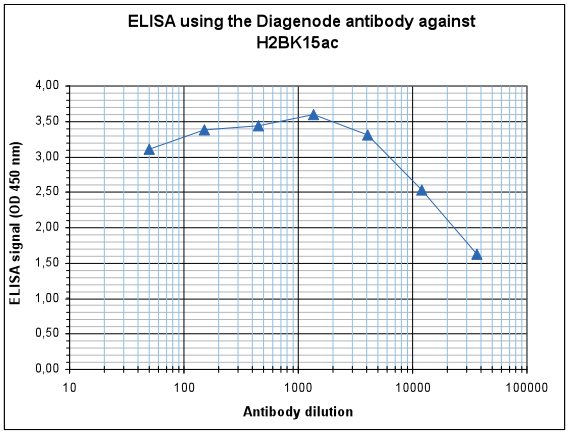
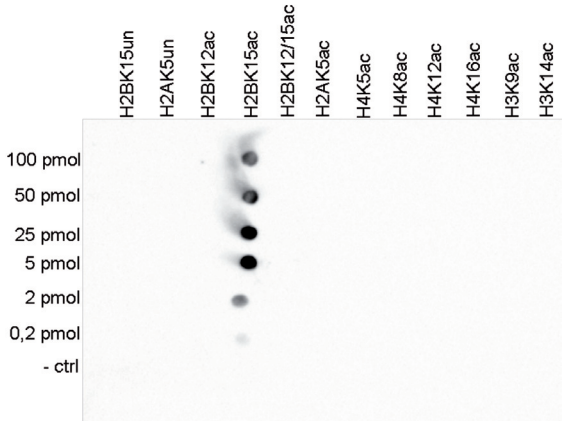


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H2BK15ac (Cat. No. C15410220) 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 3), the titer of the antibody was estimated to be 1:29,700.

Figure 4. Cross reactivity tests using the Diagenode antibody directed against H2BK15ac

To test the cross reactivity of the Diagenode antibody against H2BK15ac (Cat. No. C15410220), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H2B. 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 4 shows a high specificity of the antibody for the modification of interest.



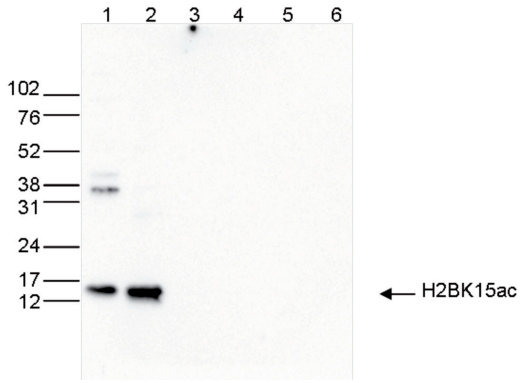
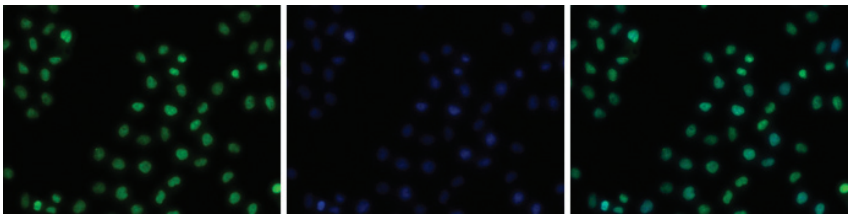


Figure 5. Western blot analysis using the Diagenode antibody directed against H2BK15ac

Western blot was performed on whole cell (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 H2BK15ac (Cat. No. C15410220). The antibody was diluted 1:500 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.

Figure 6. Immunofluorescence using the Diagenode antibody directed against H2BK15ac

HeLa cells were stained with the Diagenode antibody against H2BK15ac (cat. C15410220) 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 labeled with the H2BK15ac antibody (left) diluted 1:500 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.



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