TECHNICAL DATASHEET

LOT: 001–17

HOLOGIC® Diagenode

H3K27ac antibody

Cat. No. C15200184

Lot:	001–17
Size:	10 µg / 50 µg
Type:	Monoclonal, **ChIP-grade**, **ChIP-seq grade**, **CUT&Tag-grade**
Isotype:	IgG1
Source:	Mouse
Concentration:	1 µg/µl

Specificity:	Human, Nematodes: positive Other species: not tested
Purity:	Protein A purified monoclonal antibody
Storage buffer:	PBS containing 0.05% azide.

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: Monoclonal antibody raised in mouse against histone H3 acetylated at lysine 27 (H3K27ac), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	0.5–1 µg per ChIP	Fig 1, 2
CUT&Tag	1 µg	Fig 3
ELISA	1:3,000	Fig 4
Western blotting	1:1,000– 1:2,000	
Immunofluorescence	1:500	Fig 5

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5-5 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.

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Results

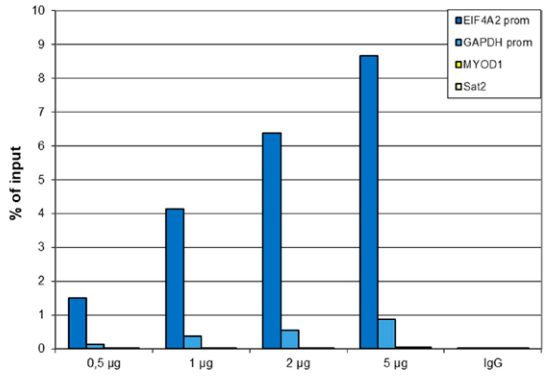

Figure 1: ChIP results obtained with the antibody directed against H3K27ac

ChIP assays were performed using human HeLa cells, the monoclonal antibody against H3K27ac (cat. No. C15200184) and optimized PCR primer pairs for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (cat. No. C01010051), using 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 EIF4A2 and GAPDH genes, used as positive controls, and for 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).

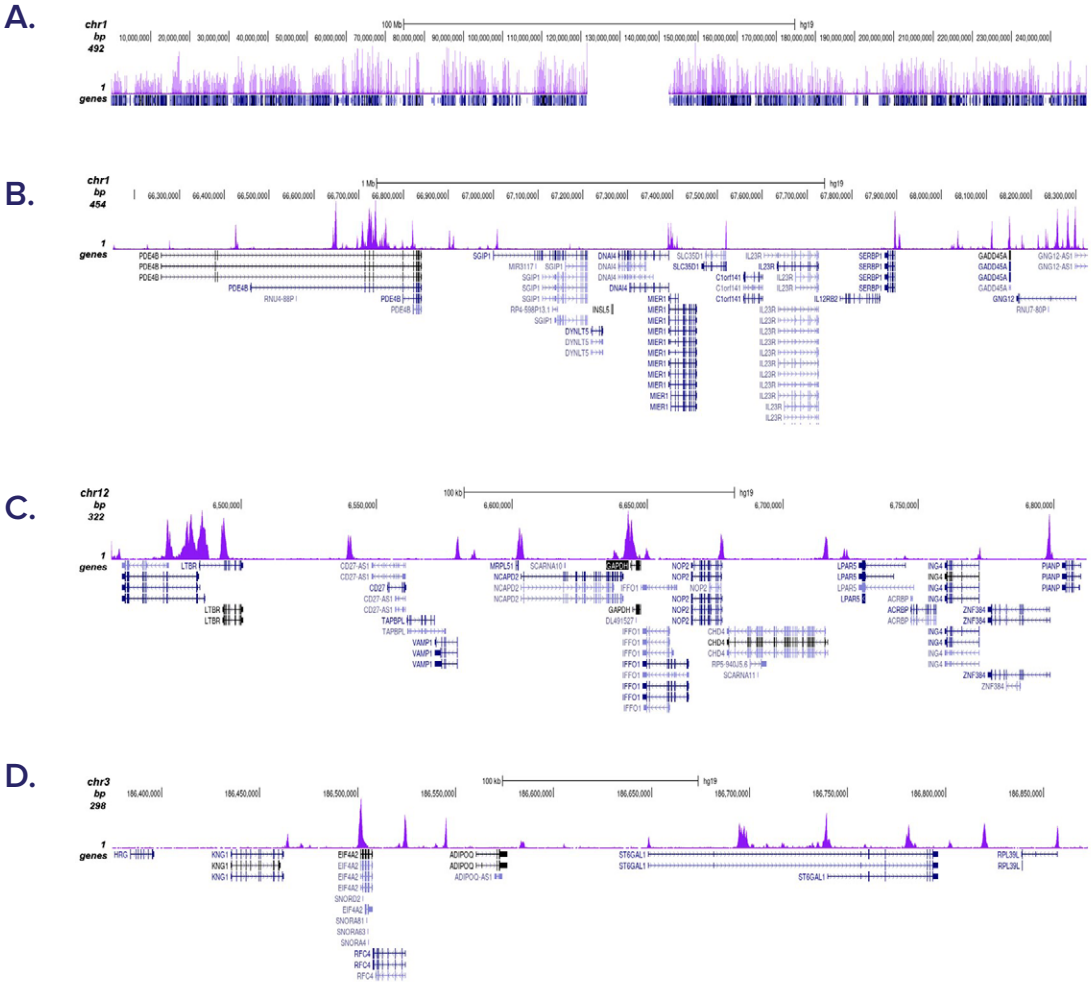

Figure 2: ChIP-seq results obtained with the antibody directed against H3K27ac

ChIP was performed on sheared chromatin from 500000 HeLa cells using 1 µg of the monoclonal antibody against H3K27ac (cat. No. C15200184) as described above. Libraries were prepared with the Microplex library preparation kit (cat. No. C05010001) and analysed on an Illumina HiSeq4000. Cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence and a 2 Mb region of chromosome 1 (figure 2A and B) and in two regions surrounding the GAPDH and EIF4A2 positive control genes, respectively (figure 2C and D).

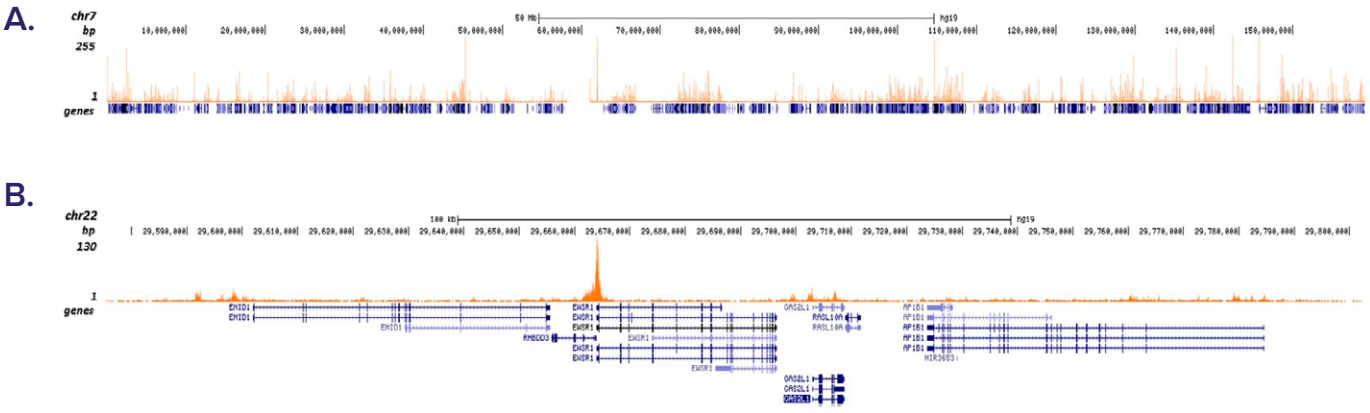

Figure 3: Cut&Tag results obtained with the antibody directed against H3K27ac

CUT&Tag was performed on 50,000 K562 cells using 1 µg of the antibody against H3K27ac (cat. No. C15200184) and the iDeal CUT&Tag kit (C01070020). The libraries were subsequently analysed on an Illumina NextSeq 500 sequencer (2x75 paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg19) using the BWA algorithm. Figure 3 shows the peak distribution along the complete sequence of chromosome 7 and in a 250 kb region surrounding the ESWR1 gene on chromosome 12 (figure 3A and B, respectively).

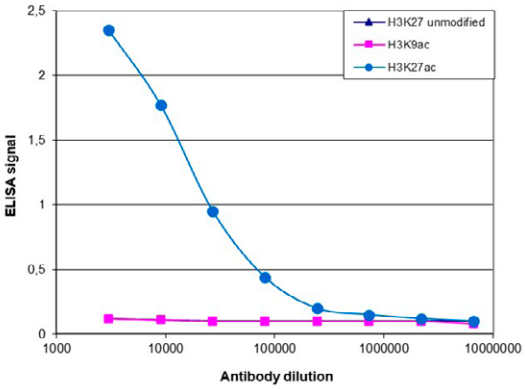

Figure 4: Cross reactivity of the antibody directed against H3K27ac

To test the specificity an ELISA was performed using a serial dilution of the monoclonal antibody against H3K27ac (cat. No. C15200184). The wells were coated with peptides containing the unmodified H3K27 region as well as the acetylated H3K27 and the acetylated H3K9. Figure 4 shows a high specificity of the antibody for the peptide containing the modification of interest.

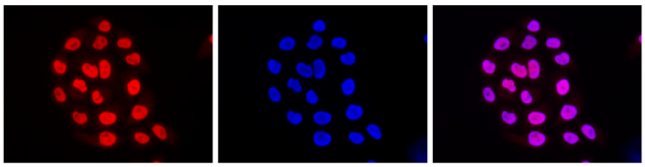

Figure 5: Immunofluorescence using the antibody directed against H3K27ac

HeLa cells were stained with the monoclonal antibody against H3K27ac (cat. No. C15200184) 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 labelled with the H3K27ac antibody (left) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.