TECHNICAL DATASHEET

LOT: 009

HOLOGIC® Diagenode

H3K27ac antibody

Cat. No. C15210016

Lot:	009
Size:	100 µg
Type:	Monoclonal, **ChIP-grade**, **ChIP-seq grade**, **CUT&Tag-grade**
Source:	Rabbit
Concentration:	1 µg/µl

Specificity:	Human, wide range expected
Purity:	Affinity purified monoclonal antibody
Storage buffer:	PBS containing 50% glycerol, 1% BSA and 0.09% 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 rabbit against the region of histone H3 containing the acetylated 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
Dot blotting	1:2,000	Fig 4
Western blotting	1:1,000	Fig 5
Immunofluorescence	1:1,000	Fig 6

*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. Acetylation of histone H3K27 is associated with active genes.

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Results

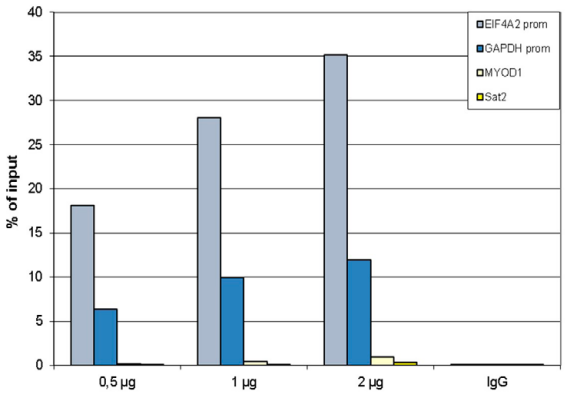

Figure 1: ChIP results obtained with the monoclonal antibody directed against H3K27ac

ChIP was performed with the antibody against H3K27ac (cat. No. C15210016) on sheared chromatin from 500,000 HeLaS3 cells using the iDeal ChIP-seq kit (cat. No. C01010051). A titration of the antibody consisting of 0.5, 1, and 2 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for the promoters of the GAPDH and EIF4A2 genes, used as positive controls, and for the MYOD1 gene and the Sat2 satellite repeat, used as negative controls. The graph shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

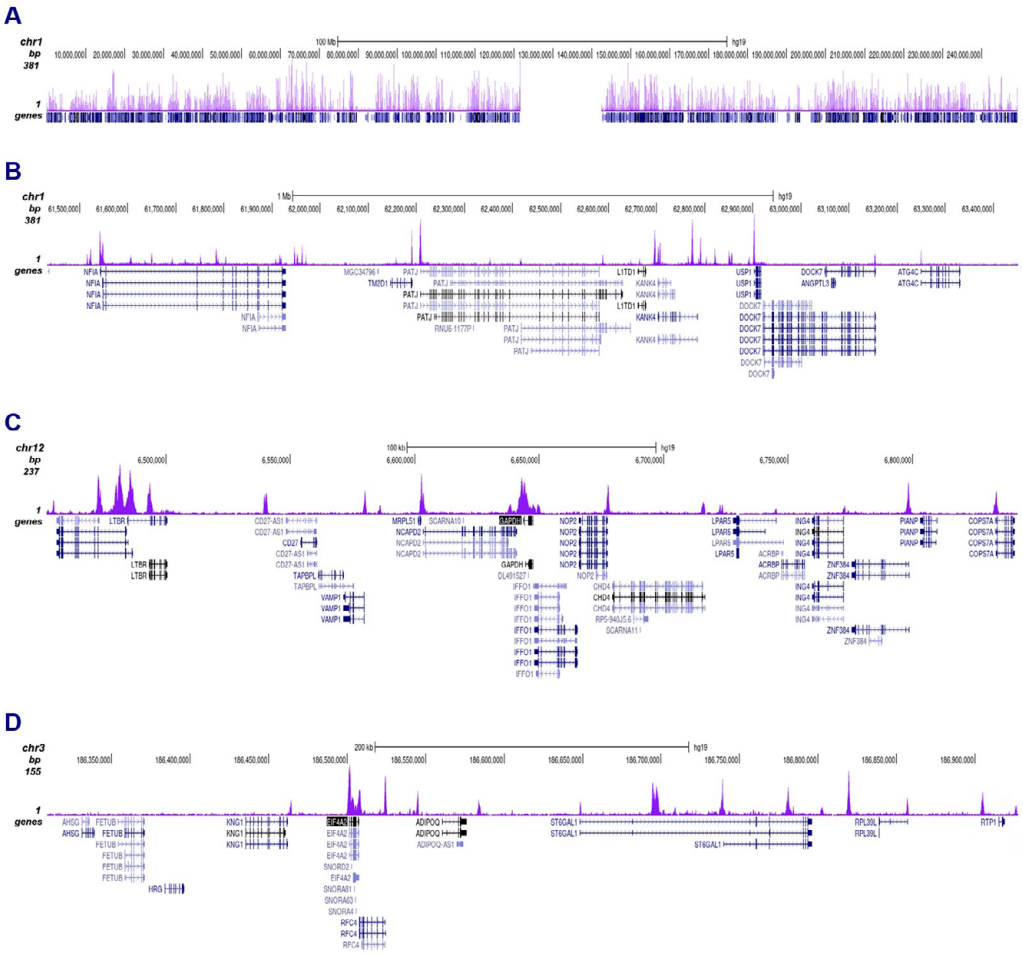

Figure 2: ChIP-seq results obtained with the monoclonal antibody directed against H3K27ac

ChIP was performed on sheared chromatin from 500000 HeLa cells using 0.5 µg of the antibody against H3K27ac (cat. No. C15210016) as described above. The IP'd DNA was subsequently analysed on an Illumina NovaSeq. Library preparation, 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 the human chromosome 1 (figure 2A and B) and in two regions surrounding the GAPDH and EIF4A2 positive control genes, respectively (figure 2C and D).

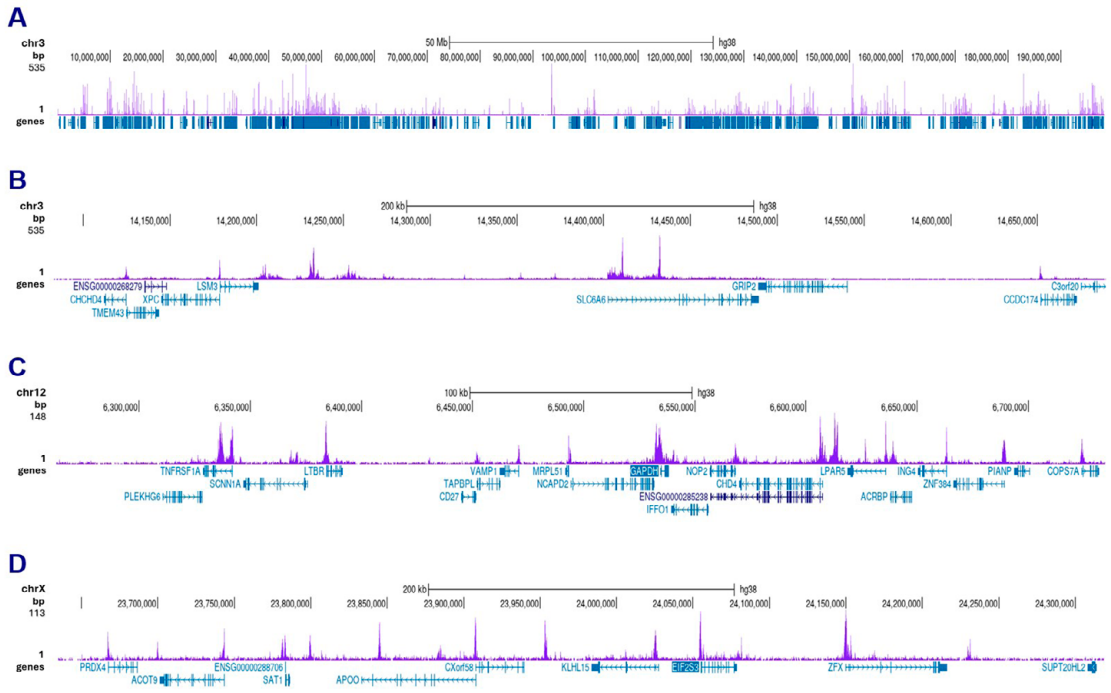

Figure 3: CUT&Tag results obtained with the monoclonal antibody directed against H3K27ac

CUT&Tag was performed on 50,000 K562 cells using 1 µg of the antibody against H3K27ac (cat. No. C15210016) and the Universal CUT&Tag kit (cat. No. C01070024). The libraries were subsequently analysed on an Illumina NovaSeqX sequencer (2x150 bp paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg38) using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence and a 600 kb region of chromosome 3 (figure 2A and B) and in 2 genomic regions on chromosome 12 and X (figure 2C and D, respectively).

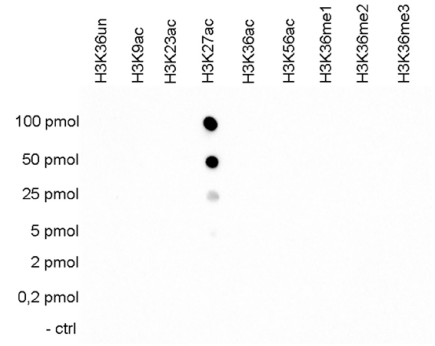

Figure 4: Cross reactivity tests using the monoclonal antibody directed against H3K27ac

To test the cross reactivity of the antibody against H3K27ac (cat. No. C15210016), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K27. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:2,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

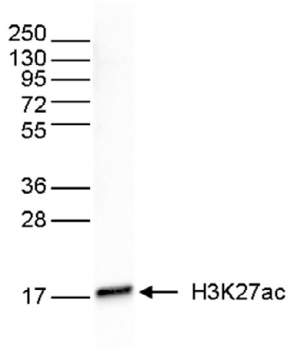

Figure 5: Western blot analysis using the monoclonal antibody directed against H3K27ac

Western blot was performed on whole cell extracts (40 µg) from HeLa cells using the antibody against H3K27ac (cat. No. C15210016). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is shown on the right, the marker (in kDa) is shown on the left.

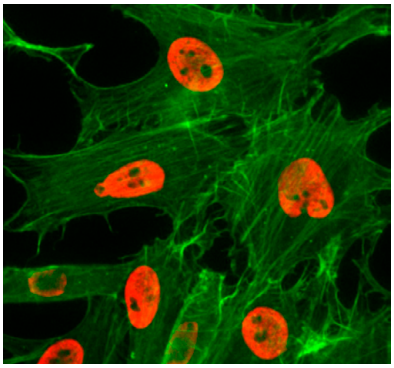

Figure 6: Immunofluorescence using the monoclonal antibody directed against H3K27ac

HeLa cells were stained with the antibody against H3K27ac (cat. No. C15210016, red) diluted 1:1,000. Actin was stained with fluorescein phalladoin (green).