TECHNICAL DATASHEET

LOT: 001

HOLOGIC® Diagenode

H4K16ac antibody

Cat. No. C15410300

Lot:	001
Size:	50 µg
Type:	Polyclonal, **CUT&Tag-grade**
Source:	Rabbit
Concentration:	1 µg/µl

Specificity:	Human, mouse, C. elegans, rat, chicken, Xenopus, Drosophila, plant: positive. Other species: not tested.
Purity:	Affinity purified polyclonal antibody
Storage buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride; contains 0.01% azide as preservative

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 histone H4 acetylated at lysine 16, using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
CUT&Tag	1 µg	Fig 1
Dot blotting	1:1,000	Fig 2
Western blotting	1:500	Fig 3
Immunofluorescence	1:100	Fig 4

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.

Diagenode, SA
BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue du Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders.diagenode@hologic.com
support.diagenode@hologic.com

Diagenode, LLC
USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

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Results

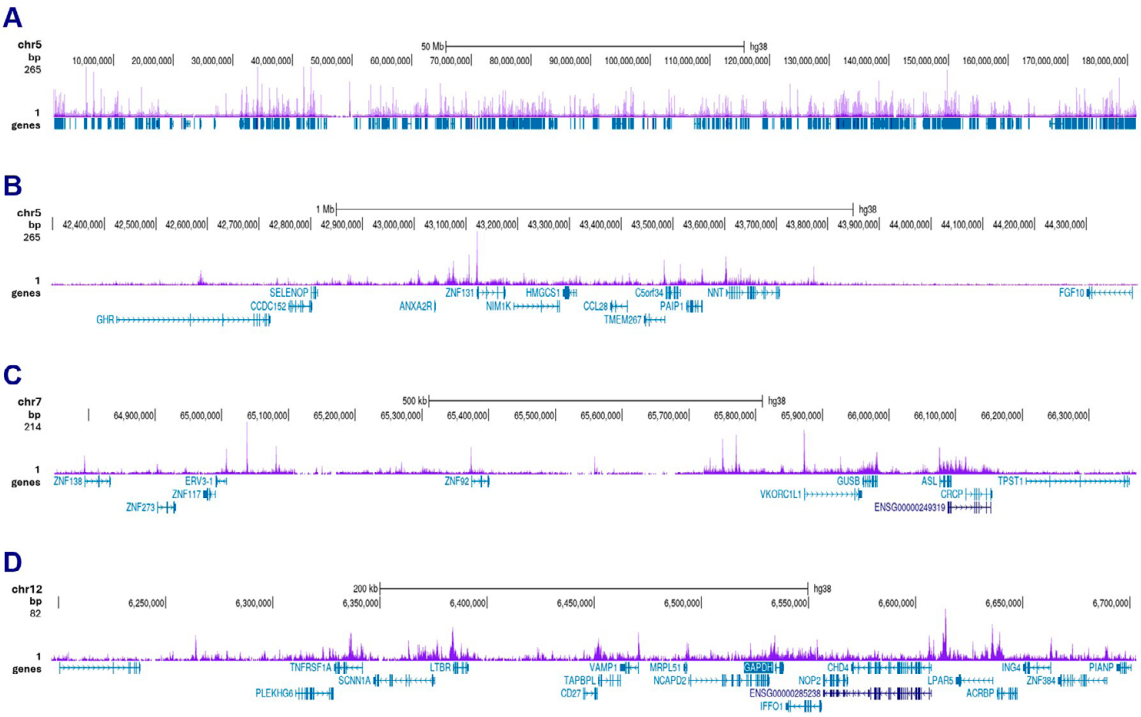

Figure 1: CUT&Tag results obtained with the antibody directed against H4K16ac

CUT&Tag was performed on 50,000 K562 cells using 1 µg of the antibody against H4K16ac (cat. No. C15410300) 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 2 Mb region of chromosome 5 (figure 1A and B) and in 2 genomic regions on chromosome 7 and 12 (figure 1C and D, respectively).

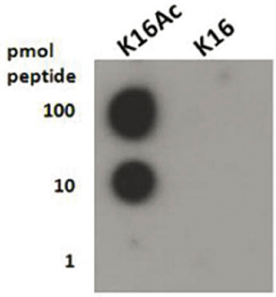

Figure 2: Cross reactivity test using the antibody directed against H4K16ac

A Dot Blot analysis was performed to test the cross reactivity of the antibody against H4K16ac (cat. No. C15410300) with peptides containing the acetylated or unmodified H4K16 sequence. One hundred to 1 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:1,000. Figure 2 shows a high specificity of the antibody for the acetylated peptide.

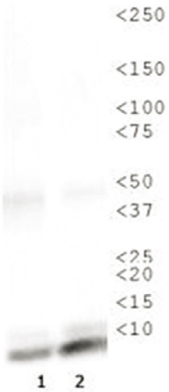

Figure 3: Western blot analysis using the antibody directed against H4K16ac

Western blot was performed on 30 µg histone extracts from HeLa (lane 1) and NIH-3T3 cells (lane 2) using the antibody against H4K16ac (cat. No. C15410300). The antibody was diluted 1:500 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the right.

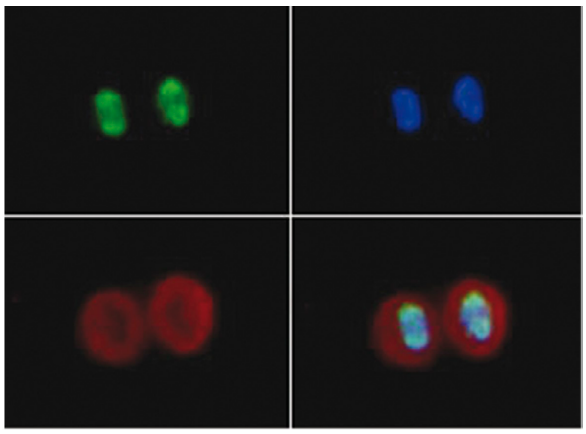

Figure 4: Immunofluorescence using the antibody directed against H4K16ac

HeLa cells were fixed with 0.5% formaldehyde and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H4K16ac antibody (upper left) diluted 1:100 in blocking solution followed by an FITC labelled anti-rabbit secondary antibody. The upper right panel shows staining of the nuclei with DAPI, whereas the lower left panel shows staining of alpha-tubulin with Dylight 594. A merge of the three stains is shown in the bottom right panel.