TECHNICAL DATASHEET

LOT: 001–13

HOLOGIC® Diagenode

H3K9ac antibody

Cat. No. C15200185

Lot:	001–13
Size:	10 µg / 50 µg
Type:	Monoclonal, **ChIP-grade**, **CUT&Tag-grade**
Isotype:	IgG2b
Source:	Mouse
Concentration:	1 µg/µl

Specificity:	Human: 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 9 (H3K9ac), using a KLH-conjugated synthetic peptide.

Applications

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

*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 protein constituents of the chromosomes in 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, 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 are dynamically regulated by histone methyl transferases and histone demethylases, respectively.

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Last update: December, 2025

Results

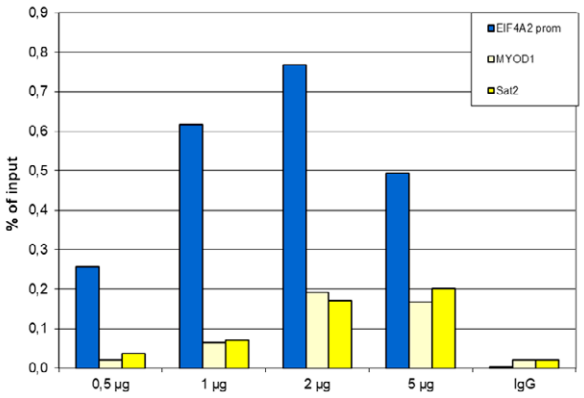

Figure 1: ChIP results obtained with the monoclonal antibody directed against H3K9ac

ChIP assays were performed using human HeLa cells, the monoclonal antibody against H3K9ac (cat. No. C15200185) and optimized PCR primer pairs for qPCR. ChIP was performed on 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 promoter of the EIF4A2 gene (used as positive control), 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).

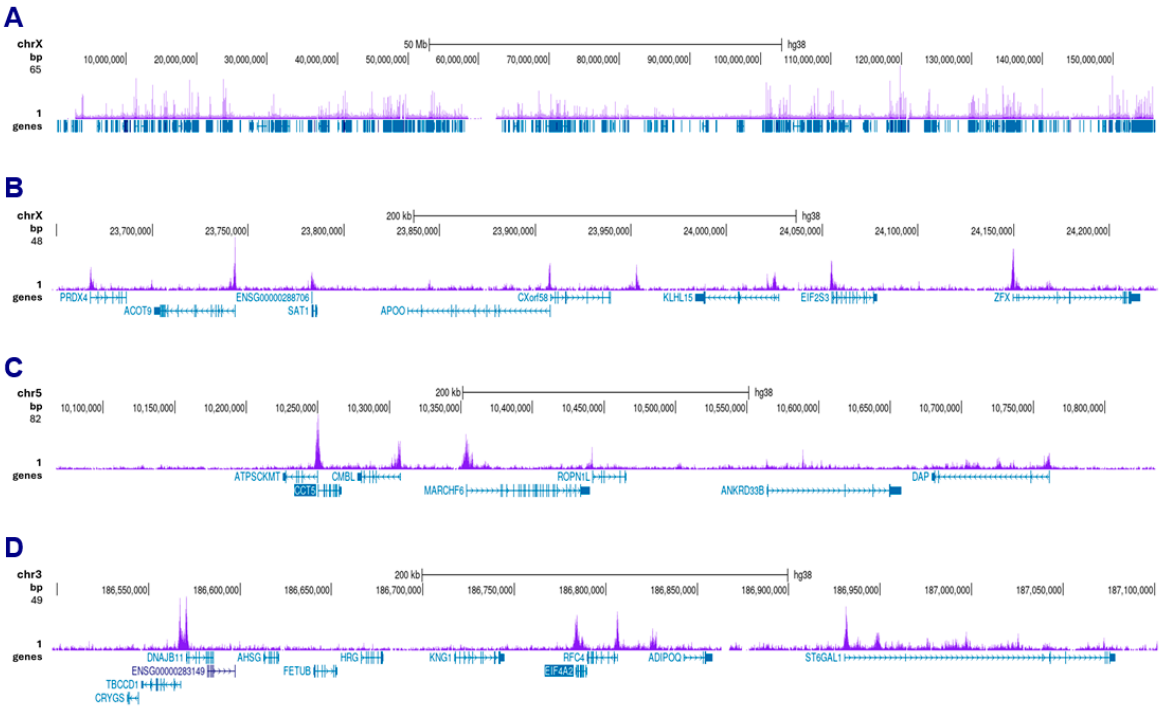

Figure 2: Cut&Tag results obtained with the monoclonal antibody directed against H3K9ac

Cut&Tag was performed on 50,000 K562 cells using 1 µg of the monoclonal antibody against H3K9ac (cat. No. C15200185), the pA-Tn5 transposase (C01070001) and the iDeal Cut&Tag kit (cat. No. C01070021). The libraries were subsequently analyzed on an Illumina NovaSeq sequencer (2x50 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 50 kb region of the X chromosome (Figure 2A and 2B), as well as in two genomic regions on chromosomes 5 and 3 (Figure 2C and 2D, respectively).

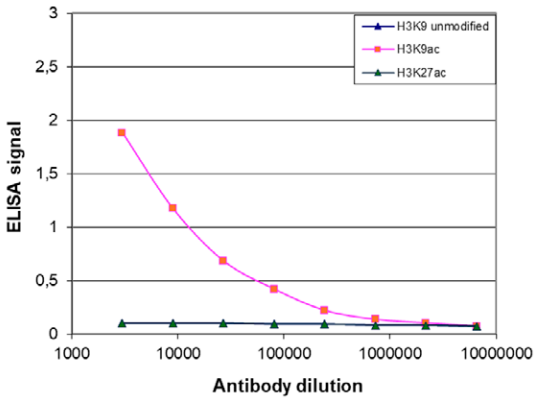

Figure 3: Cross reactivity of the monoclonal antibody directed against H3K9ac

To test the specificity, an ELISA was performed using a serial dilution of the monoclonal antibody against H3K9ac (cat. No. C15200185). The wells were coated with peptides containing the unmodified H3K9 region, as well as the acetylated H3K9 and the acetylated H3K27. Figure 3 shows a high specificity of the antibody for the peptide containing the modification of interest.

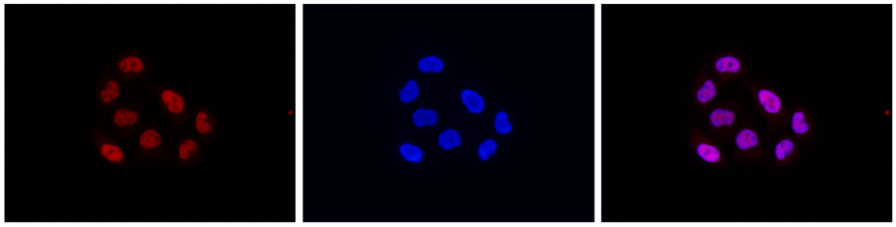

Figure 4: Immunofluorescence using the monoclonal antibody directed against H3K9ac

HeLa cells were stained with the antibody against H3K9ac (cat. No. C15200185) 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 H3K9ac 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 stains is shown on the right.