TECHNICAL DATASHEET

LOT: A156-001

HOLOGIC® Diagenode

H4K8ac antibody

Cat. No. C15310103

Lot:	A156-001
Size:	100 µl
Type:	Polyclonal, **ChIP-grade**
Source:	Rabbit
Concentration:	Not determined

Specificity:	Human: positive Other species: not tested
Purity:	Whole antiserum from rabbit containing 0.05% azide
Storage buffer:	NA

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 containing the acetylated lysine 8 (H4K8ac), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	5 µg per ChIP	Fig 1
ELISA	1:1,000 – 1:5,000	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:250	Fig 4

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 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 are dynamically regulated, respectively, by histone methyltransferases and histone demethylases. Acetylation of histone H4 is associated with active gene transcription.

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Results

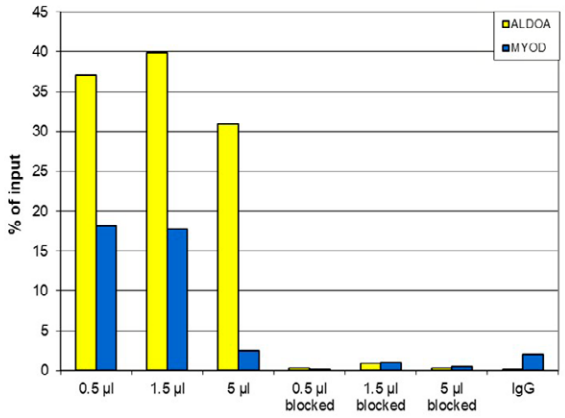

Figure 1: ChIP results obtained with the antibody directed against H4K8ac

ChIP assays were performed using human osteosarcoma (U2OS) cells, the antibody against H4K8ac (cat. No. C15310103) and optimized PCR primer sets for qPCR. ChIP was performed on sheared chromatin from 100,000 cells treated with the deacetylase inhibitor ATRA. A titration of the antibody consisting of 0.5, 1.5 and 5 µl per ChIP experiment was analysed. Additionally, ChIP was performed after incubation of the antibody with 5 nmol blocking peptide (cat. No. C16000103) for 1 hour at room temperature. IgG (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the ALDOA promoter (fructose-bisphosphate aldolase A) and for the coding region of the myogenic differentiation gene (MYOD), a gene that is inactive under normal conditions. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

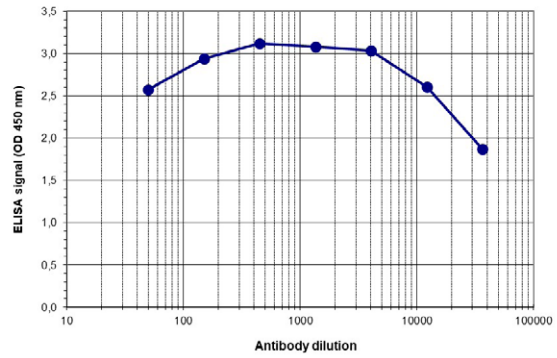

Figure 2: Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the antibody directed against human H4K8ac (cat. No. C15310103). The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), we estimated the titer of the antibody to be 1:71,500.

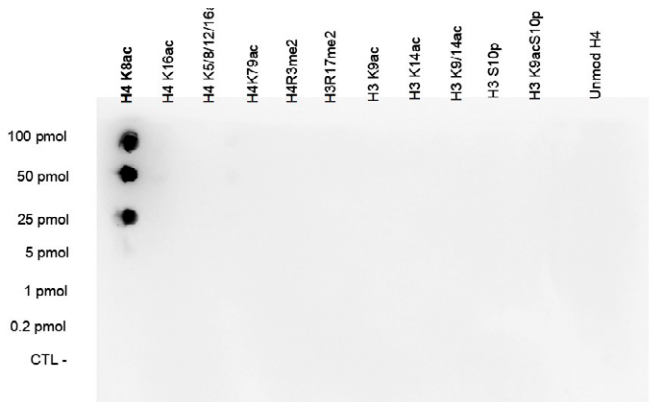

Figure 3: Cross reactivity test using the antibody directed against H4K8ac

A Dot Blot analysis was performed to test the cross-reactivity of the antibody against H4K8ac (cat. No. C15310103) with peptides containing other modifications of histone H4 and H3 and an unmodified histone H4 sequence. From 100 pmol down to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

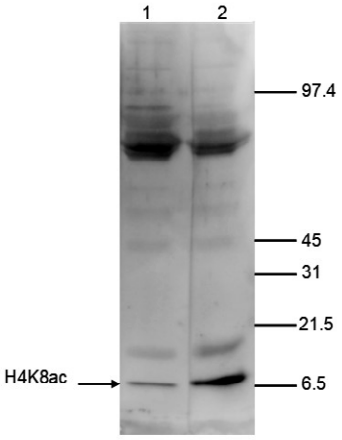

Figure 4: Western blot analysis using the antibody directed against H4K8ac

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the antibody against H4K8ac (cat. No. C15310103) diluted 1:250 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the left; the marker (in kDa) is shown on the right. Lane 2 shows the result of the Western analysis with the antibody; lane 1 shows the same analysis after incubation of the antibody with 750 pmol blocking peptide (cat. No. sp-103-050) for 1 hour at room temperature.