H3K14ac Antibody - ChIP-seq Grade

Cat. No. C15210005

Type: Monoclonal **ChIP grade, ChIP-seq grade**	Specificity: Human
Size: 100 µg	Isotype: NA
Concentration: 1 µg/µl	Host: Rabbit
Lot No.: 002	Purity: Protein A purified monoclonal antibody in PBS containing 1% BSA and 0.09% azide.
Storage buffer: NA	Storage conditions: 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.	

Last Data Sheet Update: October 20, 2021

Description

Monoclonal antibody raised in rabbit against histone **H3 acetylated at Lys14 (H3K14ac),** using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	1 µg/ChIP	Fig 1, 2
Dot blot	1:10,000	Fig 3
Western Blotting	1:2,000	Fig 4
Immunofluorescence	1:500	Fig 5

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 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 Lys14 is associated with gene activation.

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Validation Data

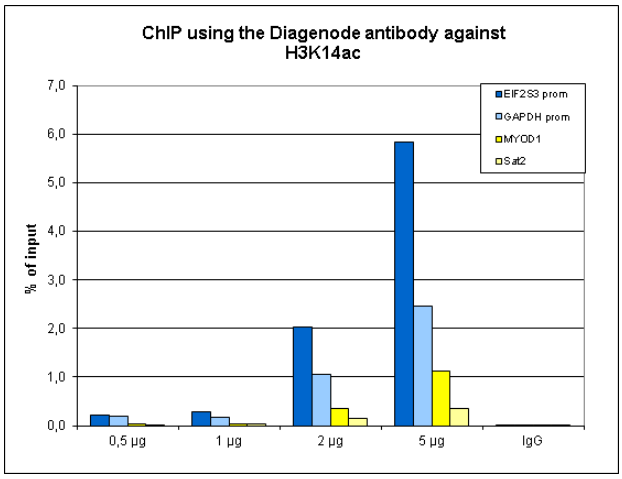
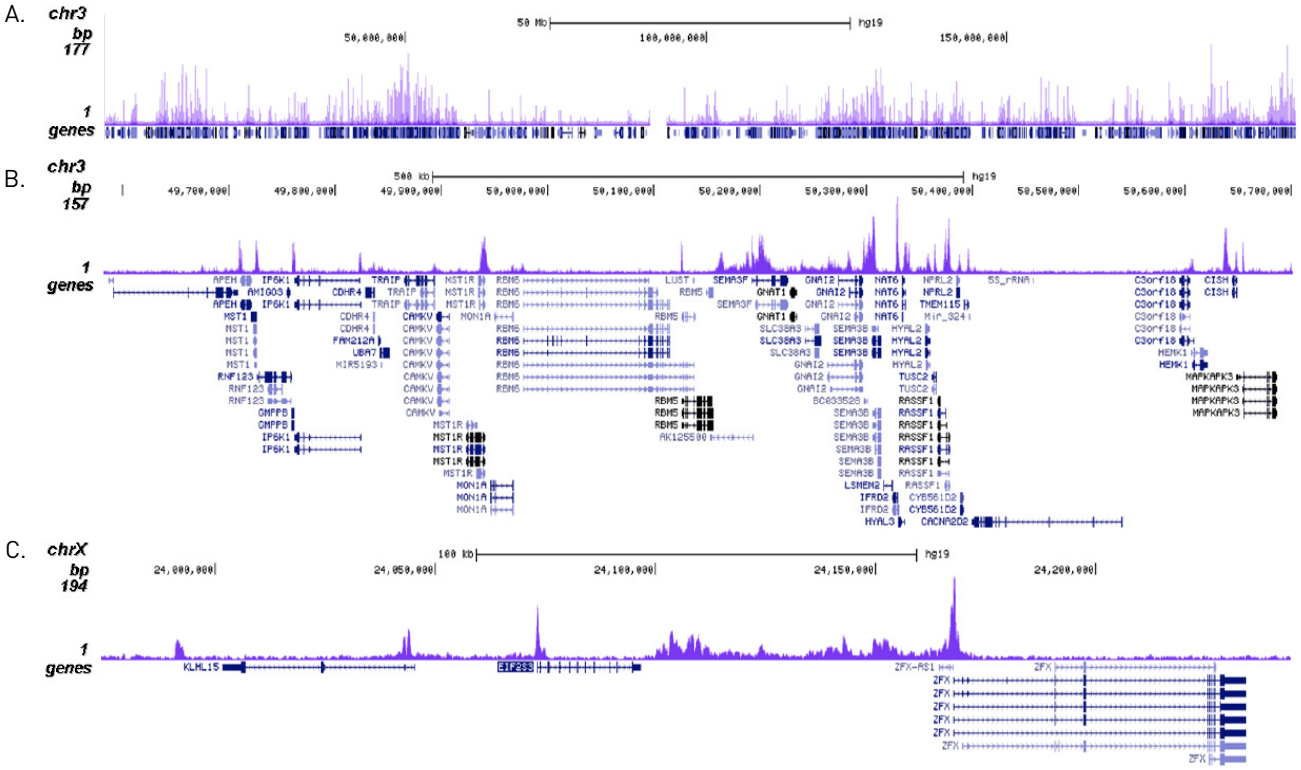


Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3K14ac

ChIP assays were performed using HeLa cells, the Diagenode antibody against H3K14ac (cat. No. C15210005) and optimized PCR primer sets 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 optimized primers for the promoters of the EIF2S3 and GAPDH genes, used as positive controls, and for the MYOD1 gene and the Sat2 satellite repeat, used as negative control. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



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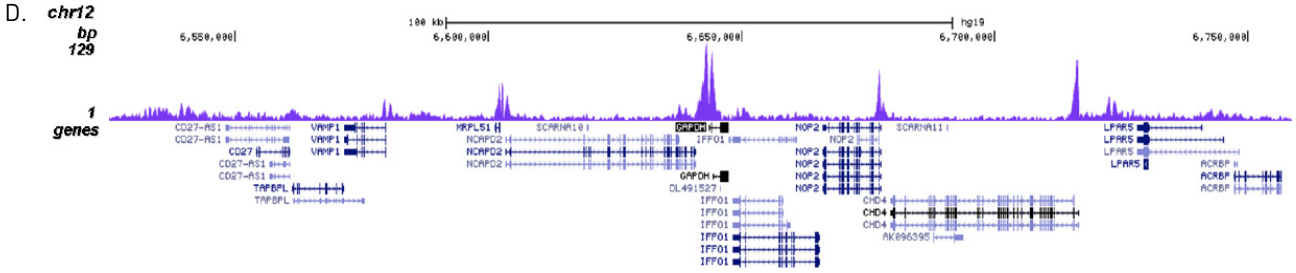

Figure 2. ChIP-seq results obtained with the Diagenode monoclonal antibody directed against H3K14ac

ChIP was performed on sheared chromatin from 1 million HeLa cells using 1 µg of the Diagenode antibody against H3K14ac (Cat. No. C15210005) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. 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 enrichment along the complete sequence and a 1 Mb region of human chromosome 3 (fig 2A and B), and in two genomic regions surrounding the EIF2S3 and GAPDH positive control genes (fig 2C and D).

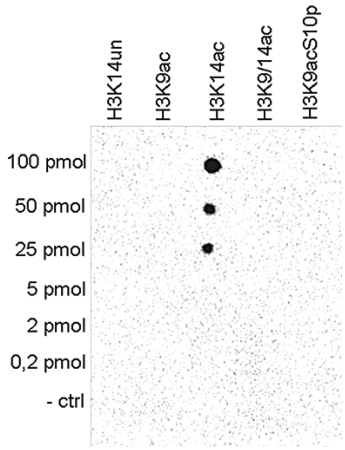

Figure 3. Cross reactivity tests using the Diagenode monoclonal antibody directed against H3K14ac

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode monoclonal antibody against H3K14ac (Cat. No. C15210005) with peptides containing different modifications or unmodified sequences of histone H3. One hundred to 0.2 pmol of peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:10,000. Figure 2 shows a high specificity of the antibody for the modification of interest.

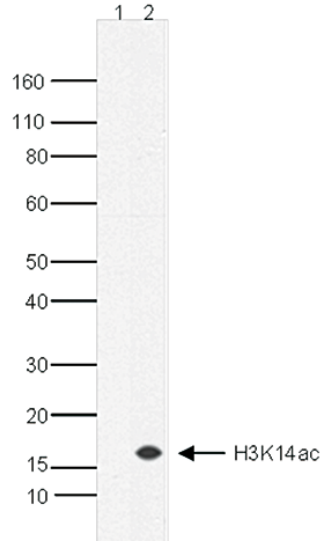

Figure 4. Western blot analysis using the Diagenode monoclonal antibody directed against H3K14ac

Histone extracts from HeLa cells treated with butyrate (lane 2) or untreated control cells (lane 1) were analysed by Western blot using the Diagenode monoclonal antibody against H3K14ac (Cat. No. C15210005) diluted 1:2,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

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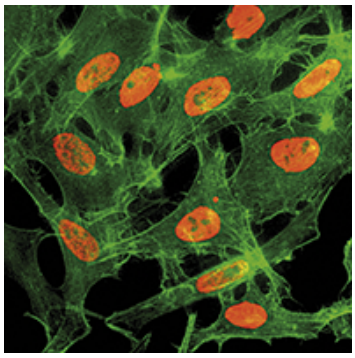

Figure 5. Immunofluorescence using the Diagenode monoclonal antibody directed against H3K14ac

HeLa cells were stained with the Diagenode antibody against H3K14ac (Cat. No. C15210005 red) diluted 1:500. Actin filamentswere stained with fluorescein phalladoin (green).

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