H3K64me3 polyclonal antibody - Classic

Cat. No. C15410211

Type: Polyclonal	Specificity: Human
Size: 50 µg	Isotype: NA
Concentration: 0.93 µg/µl	Source: Rabbit
Lot No.: A2229P	Purity: Affinity purified
Storage buffer: NA	Storage conditions: NA
Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.	

Description

Polyclonal antibody raised in rabbit against histone H3 containing the trimethylated lysine 64 (H3K64me3), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP *	1-2 µg per ChIP	Fig 1
ELISA	1:500	Fig 2
Dot Blotting	1:5,000	Fig 3
Western Blotting	1:100	Fig 4

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 ?g 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.

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Validation Data

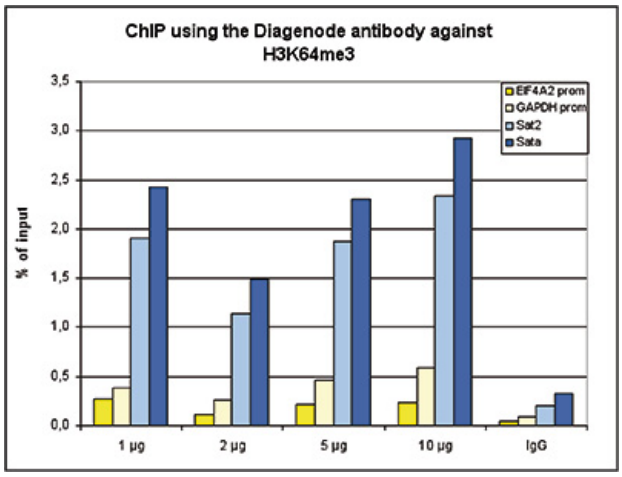

Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K64me3

ChIP assays were performed using human K562 cells, the Diagenode antibody against H3K64me3 (Cat. No. 15410211) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051) on sheared chromatin from 1 million cells. A titration of the antibody consisting of 1, 2, 5, and 10 ?g per ChIP experiment was analysed. IgG (2 ?g/IP) was used as negative IP control. QPCR was performed with primers for the promoter of the active GAPDH and EIF4A2 genes, used as negative controls, and for the Sat2 and Sata satellite repeats, used as positive controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

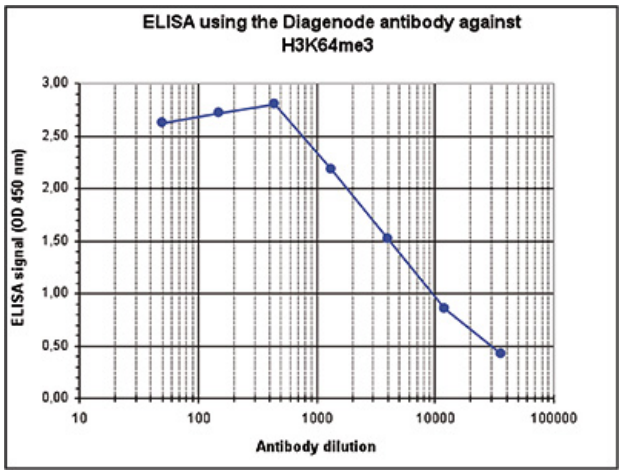

Figure 2. Determination of the antibody titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K64me3 (Cat. No. 15410211) in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:5,500.

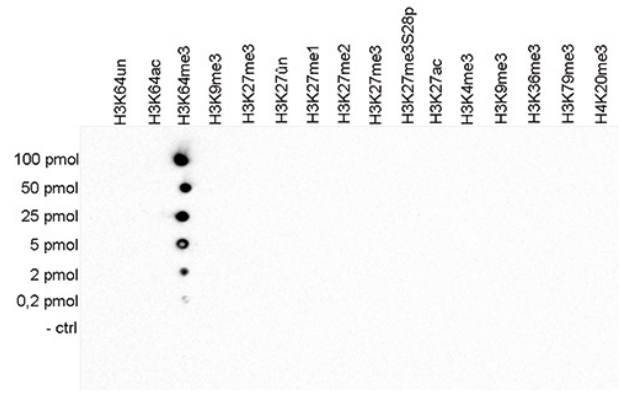

Figure 3. Cross reactivity tests using the Diagenode antibody directed against H3K64me3

To check the specificity of the Diagenode antibody against H3K64me3 (Cat. No C15410211) a Dot Blot was performed with peptides containing different modifications of histone H3 and H4 or the unmodified H3K64 sequence. 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 1:5,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

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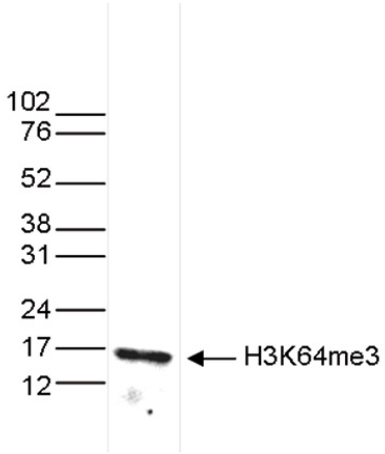

Figure 4. Western blot analysis using the Diagenode antibody directed against H3K64me3

Western blot was performed on histone extracts (30 ?g) from HeLa cells using the Diagenode antibody against H3K64me3 (Cat. No. C15410211). The antibody was diluted 1:100 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left, the position of the protein is indicated on the right.

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