TECHNICAL DATASHEET

LOT: 001-12

HOLOGIC® Diagenode

H3K36me3 antibody

Cat. No. C15200183

Lot:	001-12
Size:	10 µg / 50 µg
Type:	Monoclonal, **ChIP-grade**, **CUT&Tag-grade**
Isotype:	IgG1
Source:	Mouse
Concentration:	1 µg/µl

Specificity:	Human, rat: 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 trimethylated at lysine 36 (H3K36me3), 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 0.5-1 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 highly conserved during evolution. Histones pack DNA into tight masses of chromatin. Two core histones from each of the classes H2A, H2B, H3, and H4 assemble into an octamer, which is wrapped by 146 base pairs of DNA to form one 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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Results

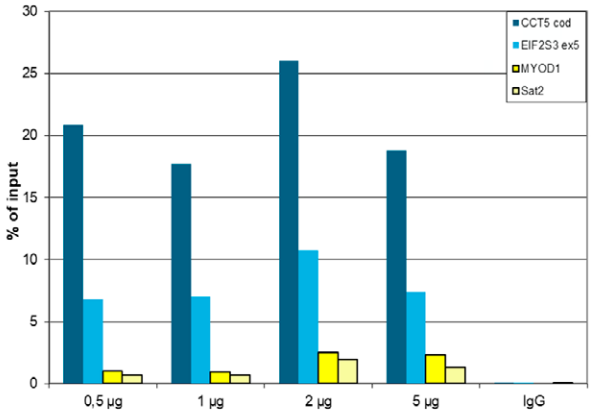

Figure 1: ChIP results obtained with the monoclonal antibody directed against H3K36me3

ChIP assays were performed using human HeLa cells, the monoclonal antibody against H3K36me3 (cat. No. C15200183), and optimized PCR primer pairs for qPCR. ChIP was performed using sheared chromatin from 1 million cells. A titration 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 coding regions of the active EIF2S3 and CCT5 genes (used as positive controls) 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).

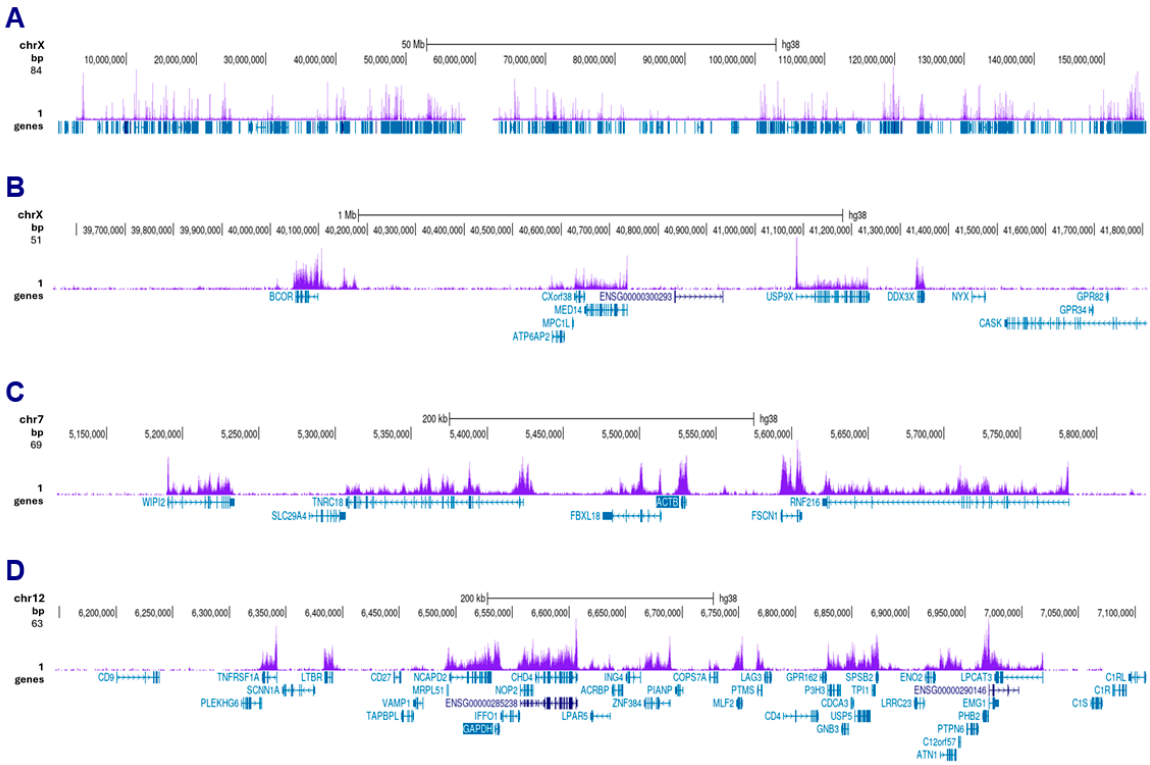

Figure 2: Cut&Tag results obtained with the monoclonal antibody directed against H3K36me3

Cut&Tag was performed on 50,000 K562 cells using 1 µg of the monoclonal antibody against H3K36me3 (cat. No. C15200183), 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 2 Mb region of the X chromosome (Figure 2A and 2B), and in two genomic regions on chromosomes 7 and 12 (Figure 2C and 2D, respectively).

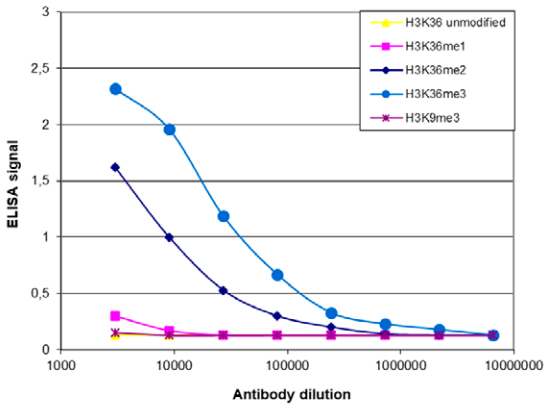

Figure 3: Cross reactivity of the monoclonal antibody directed against H3K36me3

To test the specificity, an ELISA was performed using a serial dilution of the monoclonal antibody against H3K36me3 (cat. No. C15200183). The wells were coated with peptides containing the unmodified H3K36 region as well as the mono-, di-, and trimethylated H3K36 and the trimethylated H3K9. Figure 3 shows a high specificity of the antibody for the peptide containing the modification of interest.

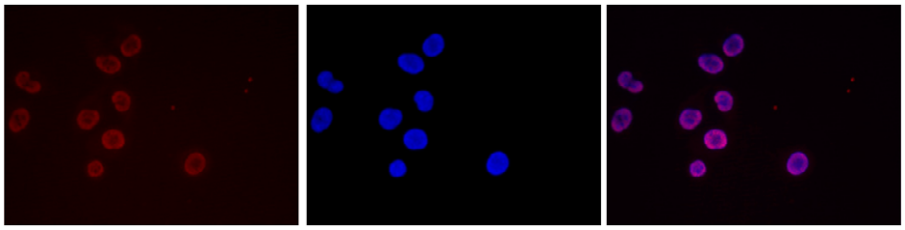

Figure 4: Immunofluorescence using the monoclonal antibody directed against H3K36me3

HeLa cells were stained with an antibody against H3K36me3 (cat. No. C15200183) and with DAPI. Cells were fixed with methanol and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labeled with the H3K36me3 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.