TECHNICAL DATASHEET

LOT: 001D

HOLOGIC® Diagenode

H3K23me2 antibody

Cat. No. C15210007

Lot:	001D
Size:	10 µg / 100 µg
Type:	Monoclonal, **ChIP-grade**, **ChIP-seq grade**, **CUT&Tag-grade**
Source:	Rabbit
Concentration:	1 µg/µl

Specificity:	Human: positive. Other species: not tested.
Purity:	Protein A purified monoclonal antibody
Storage buffer:	PBS containing 50% glycerol, 1% BSA and 0.09% 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 rabbit against histone H3 dimethylated at Lys23 (H3K23me2), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	0.5 µg per ChIP	Fig 1, 2
CUT&Tag	1 µg	Fig 3
Western blotting	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 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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Last update: May, 2026

Results

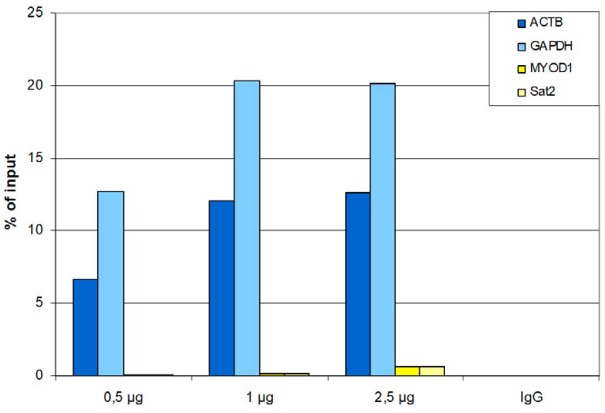

Figure 1: ChIP results obtained with the monoclonal antibody directed against H3K23me2

ChIP assays were performed using human HeLa cells, the antibody against H3K23me2 (cat. No. C15210007) and optimized PCR primer sets for qPCR. ChIP was performed with the iDeal ChIP-seq kit (cat. No. C01010055) on sheared chromatin from 1,000,000 cells. A titration of the antibody consisting of 0.5, 1 and 2.5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for a region upstream of the promoters of the ACTB and GAPDH genes, used as positive controls, and for the inactive MYOD1 gene and the Sat2 satellite repeat region 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).

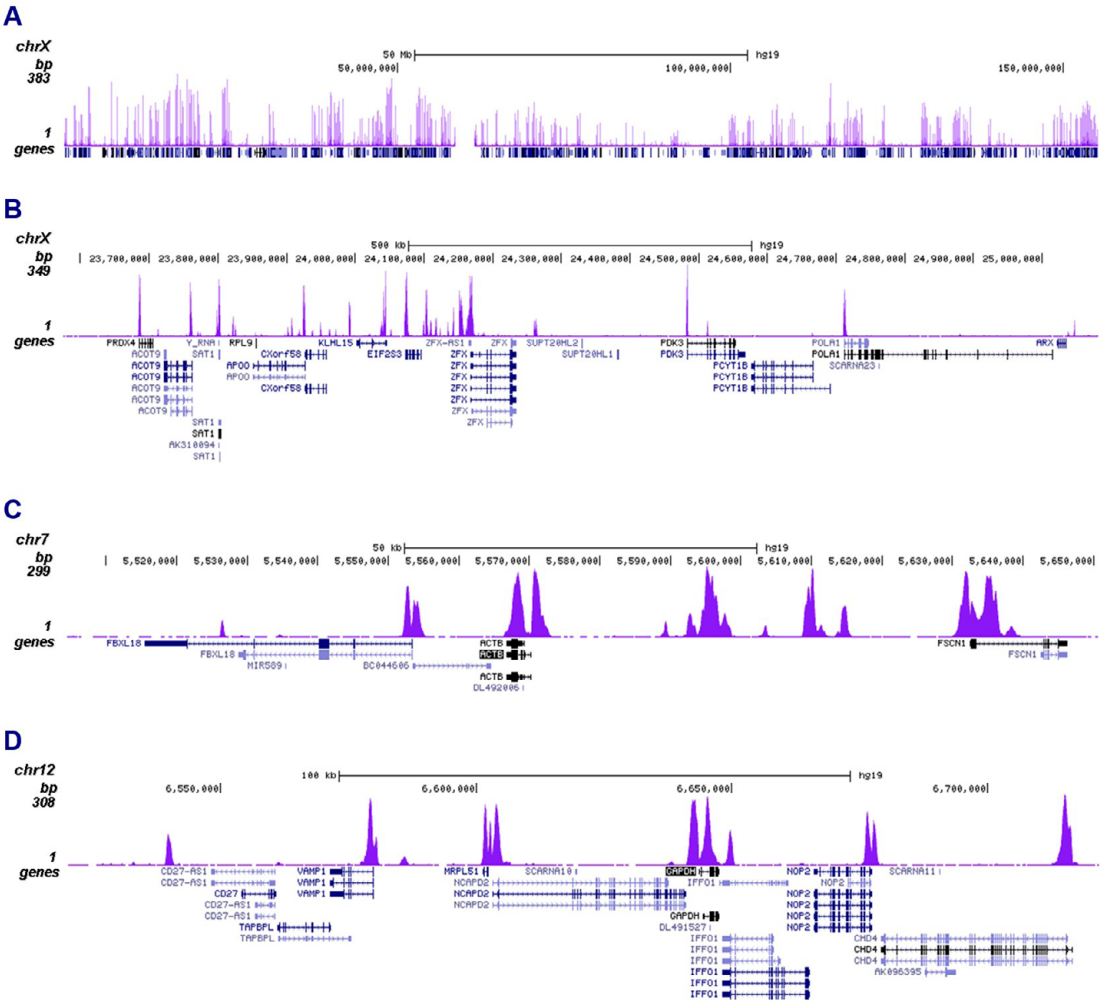

Figure 2: ChIP-seq results obtained with the monoclonal antibody directed against H3K23me2

ChIP was performed with 0.5 µg of the antibody against H3K23me2 (cat. No. C15210007) on sheared chromatin from 1,000,000 HeLa cells using the iDeal ChIP-seq kit as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. 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 peak distribution along the complete sequence and a 1.5 Mb region of the human X chromosome (figure 2A and B) and in two genomic regions surrounding the ACTB and GAPDH positive control genes (figure 2C and D).

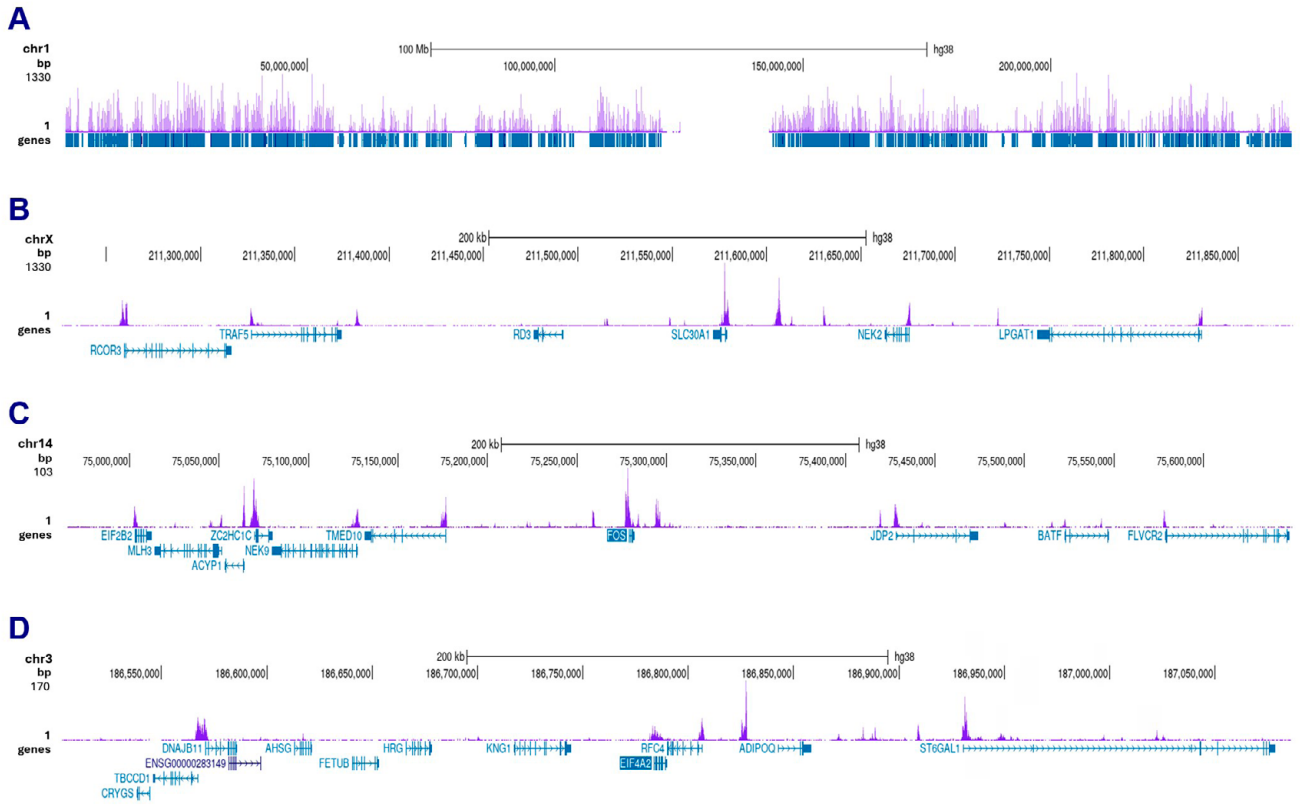

Figure 3: CUT&Tag results obtained with the antibody directed against H3K23me2

CUT&Tag was performed on 50,000 K562 cells using 1 µg of the antibody against H3K23me2 (cat. No. C15210007) and the Universal CUT&Tag kit (cat. No. C01070024). The libraries were subsequently analysed on an Illumina NovaSeqX sequencer (2x150 bp paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg38) using the BWA algorithm. Figure 3 shows the peak distribution along the complete sequence and a 600 kb region of chromosome 1 (figure 3A and B) and in 2 genomic regions on chromosome 14 and 3 (figure 3C and D, respectively).

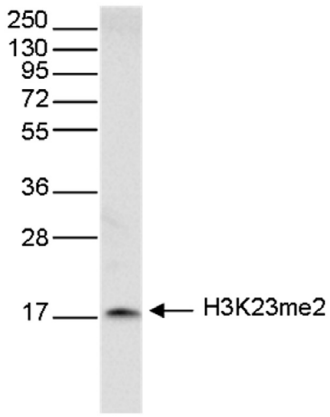

Figure 4: Western blot analysis using the monoclonal antibody directed against H3K23me2

Whole cell extracts from HeLa cells were analysed by Western blot using the monoclonal antibody against H3K23me2 (cat. No. C15210007) diluted 1:500 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.