TECHNICAL DATASHEET

LOT: A255-0010D

HOLOGIC® Diagenode

H4K20me1 antibody

Cat. No. C15410034

Lot:	A255-0010D
Size:	10 µg / 50 µg
Type:	Polyclonal, **ChIP-grade**, **CUT&Tag-grade**
Source:	Rabbit
Concentration:	2 µg/µl

Specificity:	Human: positive Other species: not tested
Purity:	Protein A purified polyclonal antibody
Storage buffer:	PBS containing 0.05% azide and 0.05% ProClin 300

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 monomethylated lysine 20 (H4K20me1), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP*	1–2 µg per ChIP	Fig 1
CUT&Tag	1 µg	Fig 2
ELISA	1:1,000	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:1,000	Fig 5
Immunofluorescence	1:500	Fig 6

*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 are dynamically regulated by histone methyltransferases and histone demethylases, respectively.

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Results

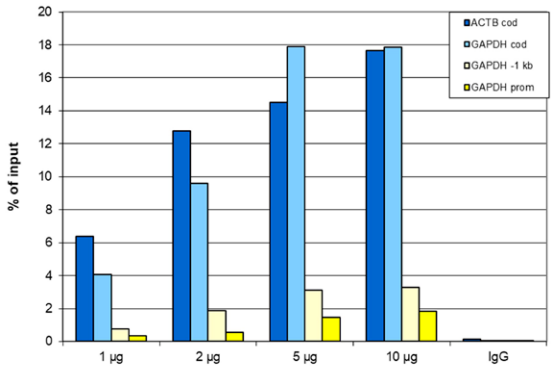

Figure 1: ChIP results obtained with the antibody directed against H4K20me1

ChIP assays were performed using human HeLa cells, the antibody against H4K20me1 (cat. No. C15410034) 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 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. qPCR was performed with primers for the coding region of the active GAPDH and ACTB genes, used as positive controls, and for the GAPDH promoter and a region located 1 kb upstream of the GAPDH promoter, 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).

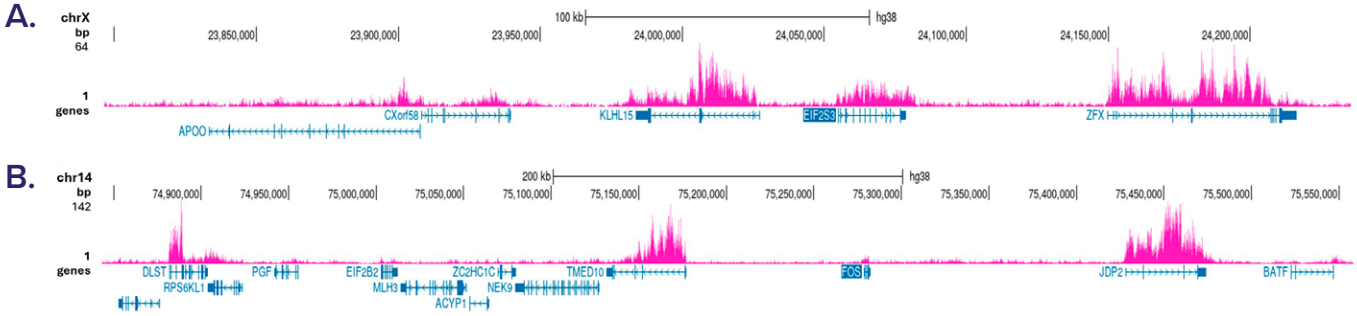

Figure 2: Cut&Tag results obtained with the antibody directed against H4K20me1

Cut&Tag was performed on 50,000 K562 cells using 1 µg of the antibody against H4K20me1 (cat. No. C15410034) and the iDeal CUT&Tag kit (C01070020). The libraries were subsequently analysed on an Illumina NovaSeq sequencer (2x50 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 in a genomic region on the X-chromosome and on chromosome 14 (figure 2A and B, respectively).

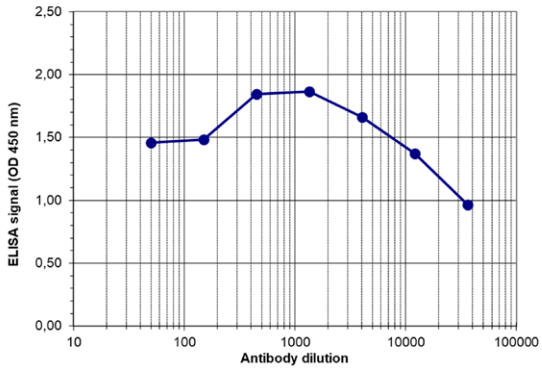

Figure 3: Determination of the titer

To determine the titer, an ELISA was performed using a serial dilution of the antibody directed against H4K20me1 (cat. No. C15410034) 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 3), the titer of the antibody was estimated to be 1:51,100.

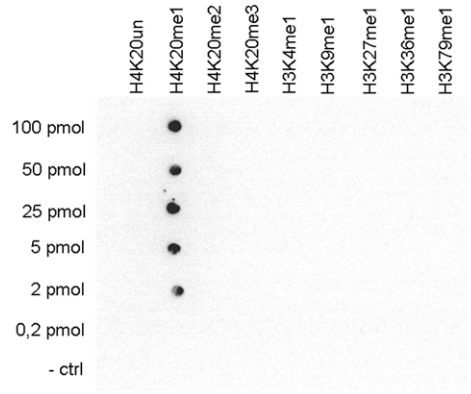

Figure 4: Cross reactivity tests using the antibody directed against H4K20me1

To check the specificity of the antibody against H4K20me1 (cat. No C15410034) a Dot Blot was performed with peptides containing different modifications of histone H3 and H4 or the unmodified H4K20 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 1:20,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

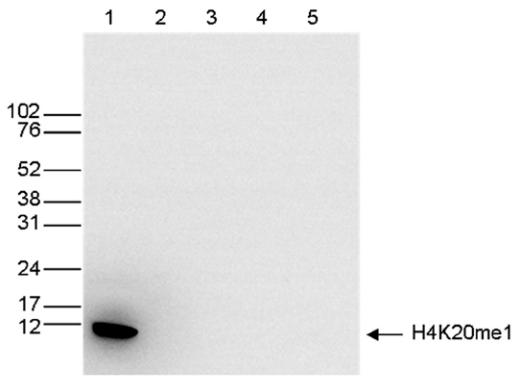

Figure 5: Western blot analysis using the antibody directed against H4K20me1

Western blot was performed on whole cell extracts (25 µg, lane 1) from HeLa cells, and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 2, 3, 4 and 5, respectively) using the antibody against H4K20me1 (cat. No. C15410034). The antibody was diluted 1:1,000 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.

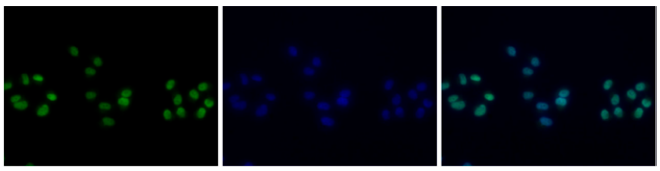

Figure 6: Immunofluorescence using the antibody directed against H4K20me1

HeLa cells were stained with the antibody against H4K20me1 (cat. C15410034) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labeled with the H4K20me1 antibody (left) diluted 1:500 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.