

PRODUCT NAME		
<b>H4K20me1 polyclonal antibody</b>		
(Histone H4 monomethylated at lysine 20)		
<b>Cat. No.</b> C15310034 (CS-034-100)	<b>Type:</b> Polyclonal <b>ChIP-grade</b>	<b>Size:</b> 100 µl
<b>Lot #:</b> A254-001	<b>Source:</b> Rabbit	<b>Concentration:</b> not determined

**Product description:** Polyclonal antibody raised in rabbit against histone H4 containing the monomethylated lysine 20 (H4K20me1), using a KLH-conjugated synthetic peptide.

**Specificity:** Human: positive  
Other species: not tested

Applications	Suggested dilution/amount	References
ChIP*	5 - 10 µl/ChIP	Fig 1
ELISA	1:300	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:750	Fig 4

\*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µl per IP.

**Purity:** Whole antiserum from rabbit containing 0.05% azide.

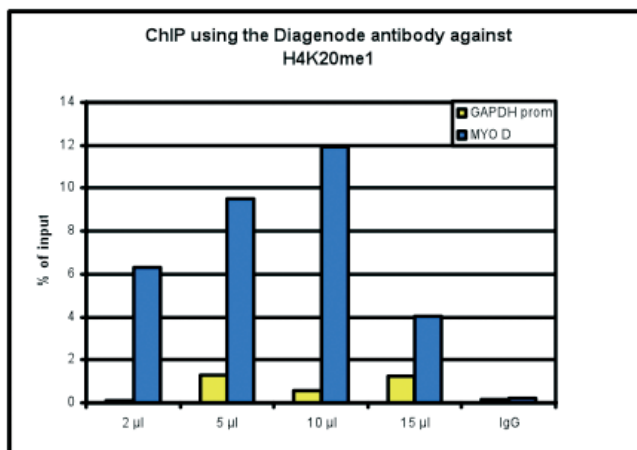
**Storage:** Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

**Precautions:** This product is for research use only. Not for use in diagnostic or therapeutic procedures.

**Last data sheet update:** April 22, 2011

**Target description**

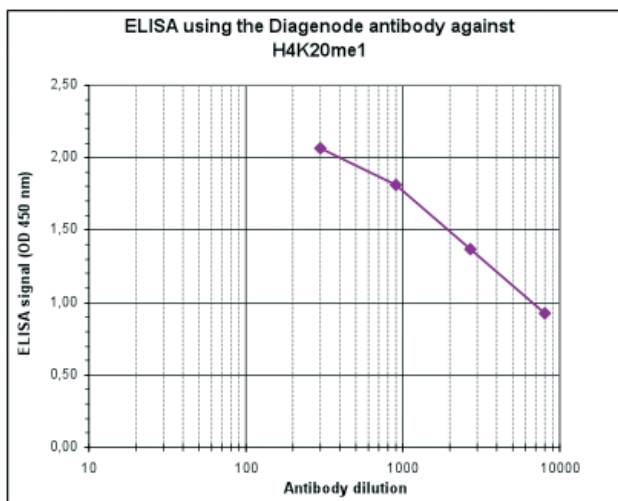
Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble 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.



**Figure 1**

**ChIP results obtained with the Diagenode antibody directed against H4K20me1**

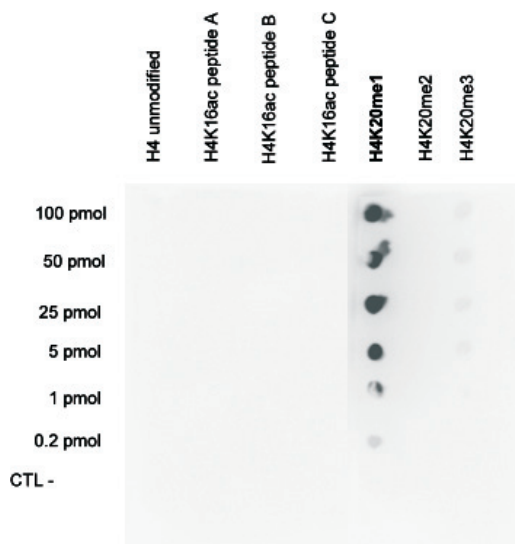
ChIP assays were performed using human osteosarcoma (U2OS) cells, the Diagenode antibody against H4K20me1 (cat. No. CS-034-100) and optimized PCR primer sets for qPCR. Chromatin was sheared with the Diagenode "Shearing ChIP" kit (cat. No. kch-redmod-100). ChIP was performed with the "OneDay ChIP" kit (cat. No. kch-oneDIP-060), using sheared chromatin from 1.6 million cells. A titration of the antibody consisting of 2, 5, 10 and 15 µl per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for the GAPDH promoter and for the coding region of the myogenic differentiation gene (MYOD), a gene that is inactive at normal conditions. 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**

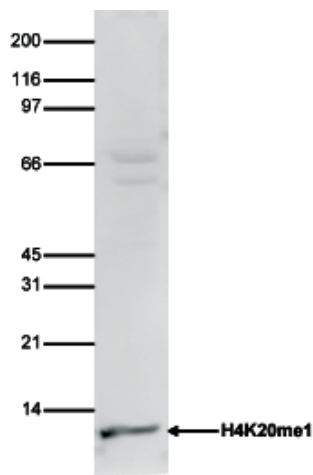
**Determination of the titer**

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody against H4K20me1 (cat. No. CS-034-100). 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 1:8,000.



**Figure 3**  
**Cross reactivity test using the Diagenode antibody directed against H4K20me1**

To check the specificity of the Diagenode antibody against H4K20me1 (cat. No. CS-034-050) a Dot Blot was performed with peptides containing other modifications of histone H4 or the unmodified sequence. Other histone modifications include mono- and dimethylation of the same lysine and acetylation of the nearby lysine 16. To determine the cross reactivity, 0.2 to 100 pmol of peptides were spotted on a membrane. Three different peptides for H4K16ac were used. The antibody was used at a dilution of 1:20,000. Figure 3 shows a high specificity of the antibody for the modification of interest.



**Figure 4**  
**Western blot analysis using the Diagenode antibody directed against H4K20me1**

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H4K20me1 (cat. No. CS-034-100) diluted 1:750 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.