TECHNICAL DATA SHEET

PRODUCT NAME H3K79me1 polyclonal antibody		
Cat. No. C15310082 (CS-082-100)	**Type:** Polyclonal **ChIP-grade**	**Size:** 100 µl
Lot #: A82-001	**Source:** Rabbit	**Concentration:** not determined

Product description: Polyclonal antibody raised in rabbit against histone H3 the monomethylated lysine 79 (H3K79me1), using a KLH-conjugated synthetic peptide.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ChIP*	5 – 10 µl/ChIP	Fig 1
ELISA	1:500 – 1:1,000	Fig 2
Dot blotting	1:100,000	Fig 3
Western blotting	1:1,000	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.

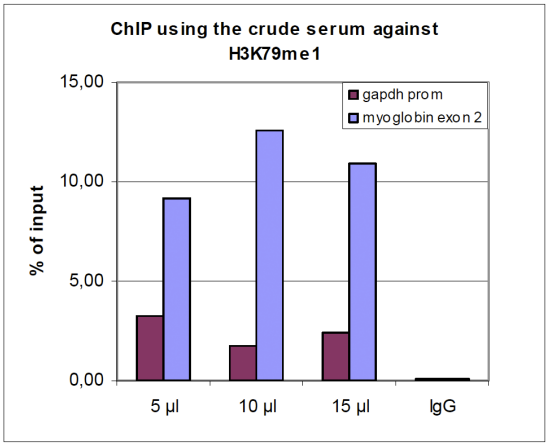
Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

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.

A



B

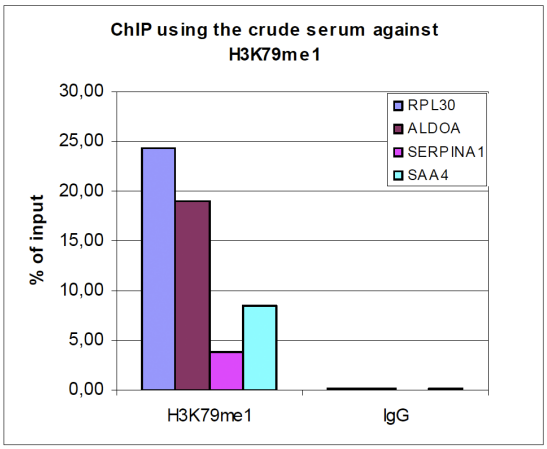


Figure 1
ChIP results obtained with the Diagenode antibody directed against H3K79me1
ChIP assays were performed using HeLa cells, the Diagenode antibody against H3K79me1 (cat. No. CS-082-100) and optimized PCR primer pairs for qPCR. Chromatin from 1.6 million cells was sheared with the Diagenode "Shearing ChIP" kit (cat. No. kch-redmod-100). ChIP was performed with the "OneDay ChIP" kit (cat. No. kch-oneDIP-060). IgG (5 µg/IP) was used as a negative IP control. The IP'd DNA was analysed by qPCR using primers for different positive and negative loci. The results are expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

Figure 1A: recovery of the GAPDH promoter and myoglobin exon 2 with a titration of the H3K79me1 antibody consisting of 5, 10 and 15 µl per ChIP experiment.

Figure 1B: recovery of RPL30, ALDOA, SERPINA1 and SAA4 using 10 µl of antibody per ChIP experiment.

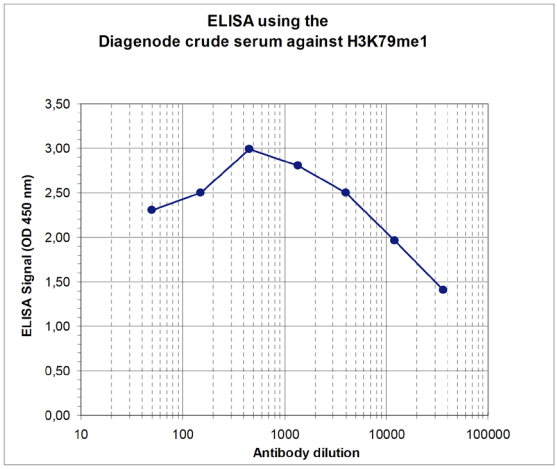


Figure 2

Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Diagenode antibody against H3K79me1 (cat. No. CS-082-100) 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 1:30,000.

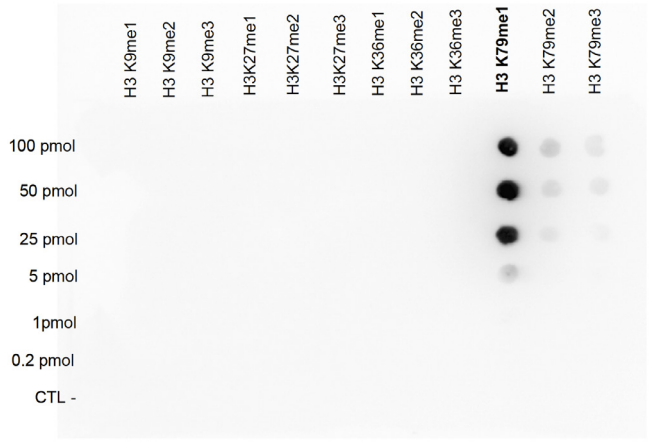


Figure 3

Cross reactivity test of the Diagenode antibody directed against H3K79me1

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K79me1 (cat. No. CS-082-100) with peptides containing other modifications of histone H3. These include di- and trimethylation of the same lysine and mono-, di- and trimethylation of lysine 9, 27 and 36. One hundred to 0.2 pmol of the peptides were spotted on a membrane. The antibody was used at a dilution of 1:100,000. Figure 3 shows a high specificity of the antibody for the modification of interest.

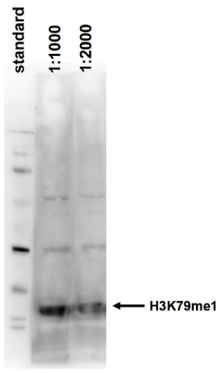


Figure 4

Western blot analysis using the Diagenode antibody directed against H3K79me1

Western blot was performed on histone extracts from HeLa cells (15 µg) with the Diagenode abtibody against H3K79me1 (cat. No. CS-082-100), diluted 1:1,000 and 1:2000 in TBS-Tween containing 5% skimmed milk. The molecular weight marker (Bio-Rad, broad range biotinylated SDS-PAGE standard) is shown on the left, the location of the protein of interest is indicated on the right.