

**Diagenode sa**  
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**Product name:**  
**crude serum directed against H4K20me3**  
**(Histone H4 [trimethylated lysine 20])**

<b>Catalog #:</b> CS-057-050	<b>Type:</b> Polyclonal <b>ChIP-grade</b>	<b>Size:</b> 50 µl
<b>Lot #:</b> A9-002	<b>Source:</b> Rabbit	<b>Concentration:</b> Not determined

**Description:** This antiserum has been raised against the region of the histone H4 containing the trimethylated lysine 20 (or [K20me3]), using a KLH-conjugated synthetic peptide.

Methylation of histone H4K20 is involved in gene repression (see overview below).

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

Applications	Suggested dilution	References
ELISA	Titer: 1:700 (crude)	Fig 1
Dot blotting	1:1,000	Fig 2
Western blotting	1:750	Fig 3
Gel Supershift	Not tested	
Immunocytochemistry	Not tested	
Flow cytometry	Not tested	
Immunoprecipitation	Not tested	
ChIP	1:100	Fig 4

**Format:** Crude rabbit serum containing 0.05% azide.

**Storage:** For long storage, store at -20°C/ -80°C. Avoid multiple freeze-thaw cycles.

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

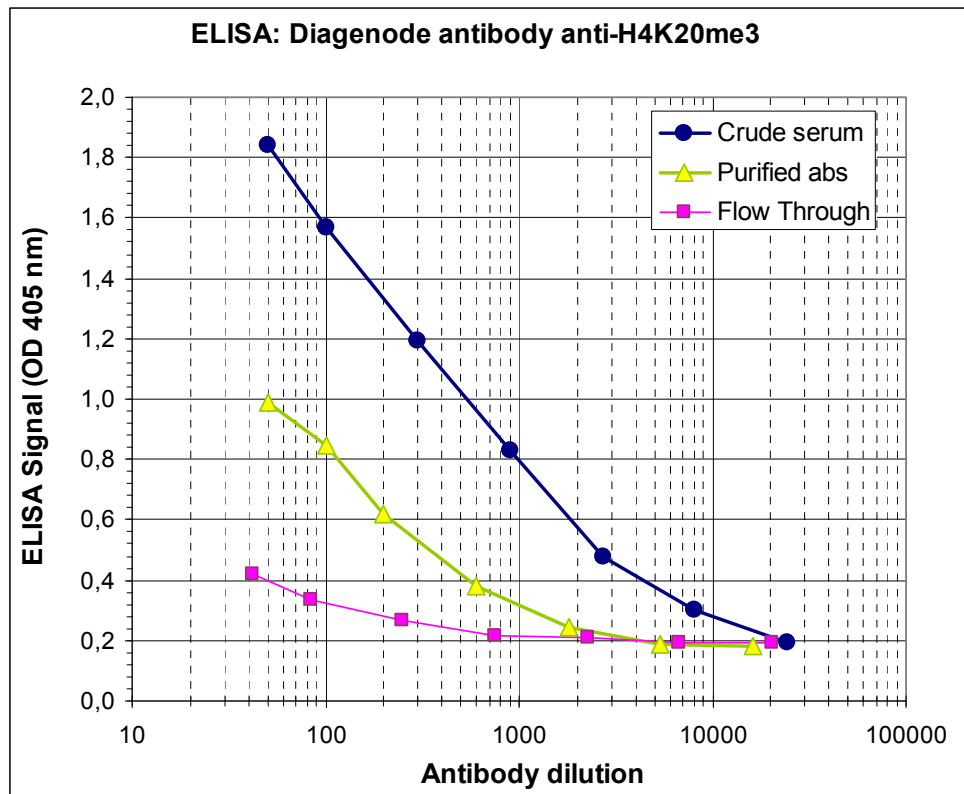
**References:**

- [1] Strahl B. and Allis C. 2000. *Nature* 403 (6765):41-5.
- [2] Zheng C. and Hayes J.J. 2003. *J Biol Chem.* 278(26):24217-24.
- [3] Shi Y. and Whetstine J.R. 2007. *Mol Cell.* 25(1):1-14.
- [4] Jenuwein T. and Allis C. 2001. *Science* 293 (5532):1074-80.

**Availability date:** June 27, 2007. Last data sheet update: June 29, 2007

**Lot #:** A09-002: rabbit #: A9/ bleed #: Day 87/ crude serum

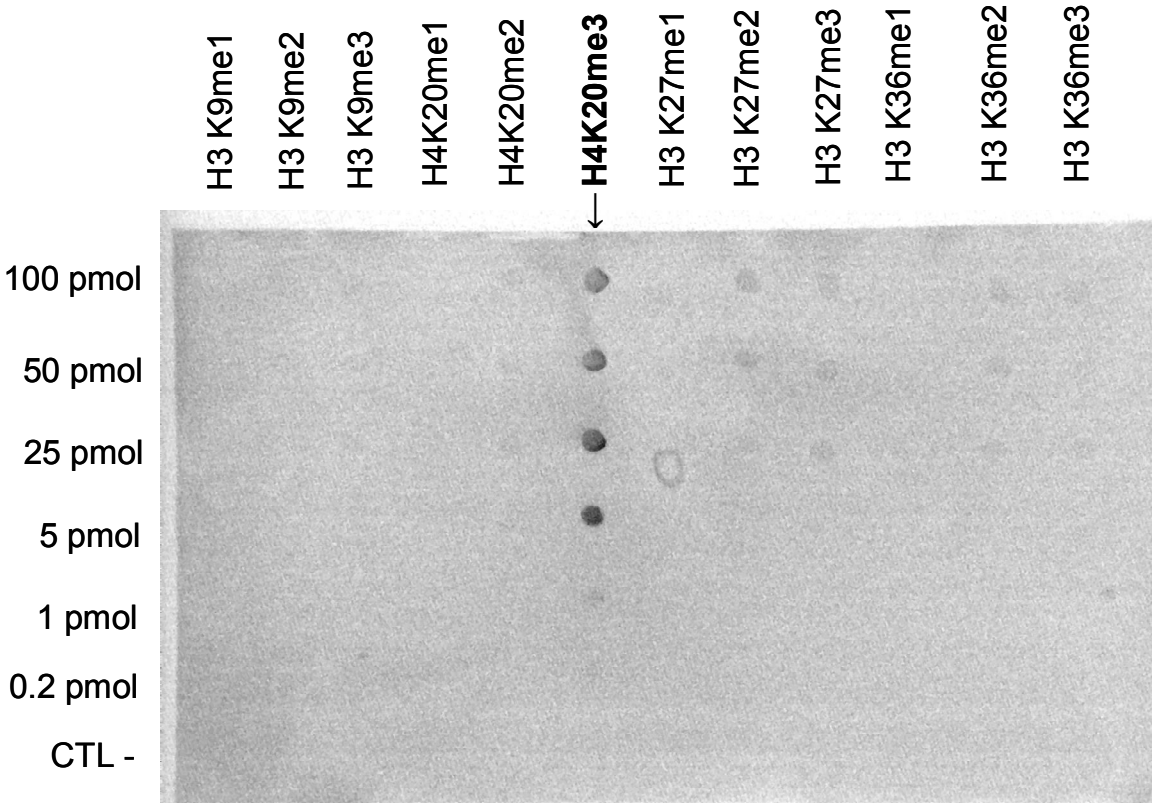
**Figure 1**



### Antibody titer

ELISA, which stands for Enzyme-Linked Immunosorbent Assay, is a quantitative method used to determine the concentration of a primary antibody using a series of dilutions of crude sera (cat# CS-057-050), affinity purified antibody (cat# pAb-057-050) and flow-through in antigen coated wells. The antigen used in this case is the peptide including the histone modification of interest. We plotted the absorbance versus antibody dilution to estimate the TITER: 1:700 for crude serum (cat# CS-057-050) and 1:350 for affinity purified antibody (cat# pAb-057-050).

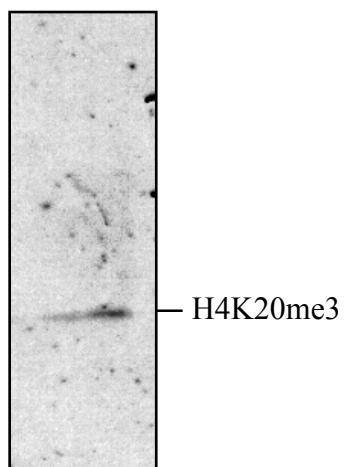
**Figure 2**



**Cross reactivity tests using the Diagenode antibody directed against H4K20me3.**

Dot Blot was used to check the specificity of the Diagenode antibody anti-H4K20me3 (cat# CS-057-050) with other histone modifications of histone H3 and histone H4. Other histone modifications include mono- and dimethylation of the same lysine and mono-, di- and trimethylation of adjacent lysines. To determine the cross reactivity, 0.2 to 100 pmol of peptide containing the respective histone modifications were spotted on a membrane. The crude serum was used at dilution 1:1,000. At this dilution, the limit of detection of the antibody is 5 pmol.

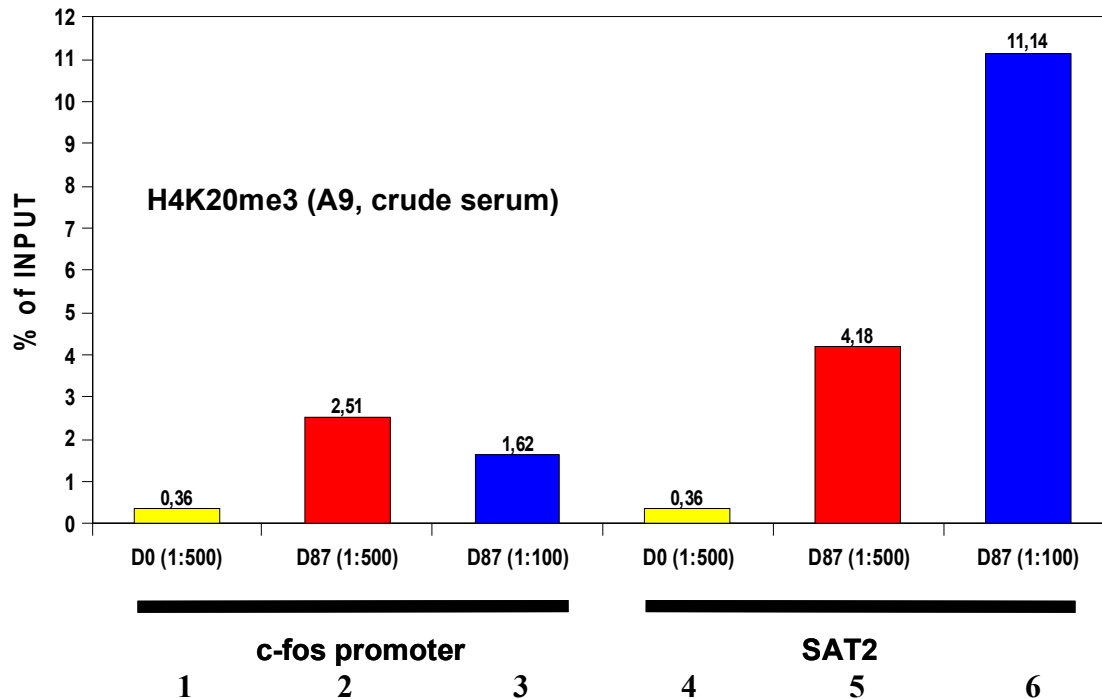
**Figure 3**



**Western blot analysis using the Diagenode crude antiserum anti-H4K20me3**

Histone (acid) extracts of NB4 cells were analysed by Western blot using the Diagenode crude serum directed against anti-H4K20me3 (cat#: CS-057-050) at a dilution of 1:750 in TBS-Tween + 5% skimmed milk. The location of the protein of interest is indicated.

**Figure 4**



**ChIP results obtained with the Diagenode crude serum directed against H4K20me3.**

ChIP assays were performed using undifferentiated human teratocarcinoma cells (NCCIT), the Diagenode crude serum directed against H4K20me3 (cat# CS-057-050) and optimized PCR primer sets for PCR. Chromatin sheared from 10,000 cells were used per ChIP experiment. Pre-immune serum (yellow bars) and crude serum (red and blue bars) were tested. H3K9me3 is a marker for heterochromatin. Therefore, we used the promoter of a house keeping gene c-fos, which is under active transcription, as negative PCR control. SAT 2, present in heterochromatin, is used as positive PCR control.

**In red:** recovery (% of input) for the antiserum H4K20me3 (D87 = day 87 of the immunization program) used at dilution 1:500, using primer pairs for negative PCR control (bar 2) and positive PCR control (bar 5).

**In blue:** recovery (% of input) for the antiserum H4K20me3 (D87) used at dilution 1:100, using primer pairs for negative PCR control (bar 3) and positive PCR control (bar 6).

**In yellow:** recovery (% of input) for the pre-immune serum (D0) using primer pairs for negative PCR control (bar 1) and positive PCR control (bar 4).

The % of recovery represents the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analyses. The antibody was titrated and used for ChIP at dilution 1:500 and 1:100, whereby the best results were obtained with dilution 1:100.

## Overview

Histones are molecules found in the chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones complex with the DNA and 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 [1,2]. Histone methylation and demethylation is dynamically regulated by respectively histone methyl transferases and histone demethylases [3]. Combinations of modifications are thought to constitute a code, the so-called "histone code" [4].