

PRODUCT NAME		
H3K79me3 polyclonal antibody		
Cat. No. <b>C15310068</b> (CS-068-100)	Type: Polyclonal	Size: 100 µl
Lot #: A86-001	Source: Rabbit	Concentration: not determined

**Description:** Polyclonal antibody raised in rabbit against histone H3 containing the trimethylated lysine 79 (H3K79me3), using a KLH-conjugated synthetic peptide.

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

Applications	Suggested dilution	References
ELISA	1:500 – 1:1,000	Fig 1
Dot blotting	1:50,000	Fig 2
Western blotting	1:500	Fig 3
Immunofluorescence	1:200	Fig 4

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

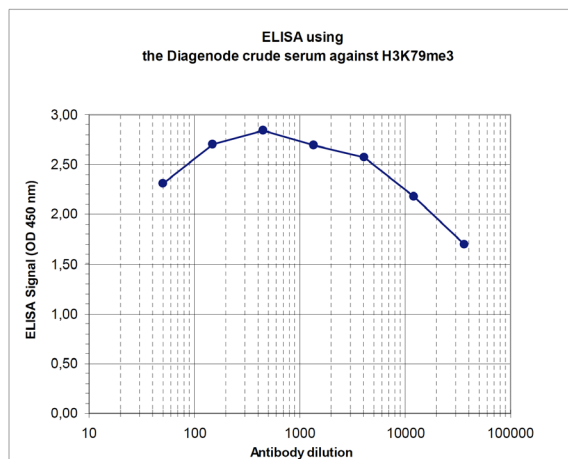
**Storage:** Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

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

**Last data sheet update:** March 11, 2010

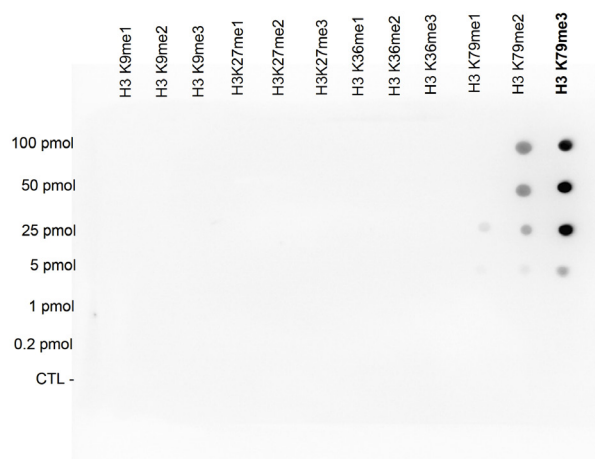
**Target description**

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. Trimethylation of histone H3 on K79 was shown to be more present at active promoters than at silent promoters.



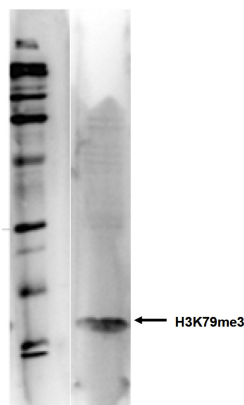
**Figure 1**  
**Determination of the antibody titer**

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Diagenode antibody directed against H3K79me3 [Cat. No. CS-068-100] 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 1), the titer of the antibody was estimated to be 1:70,000.



**Figure 2**  
**Cross reactivity test of the Diagenode antibody directed against H3K79me3**

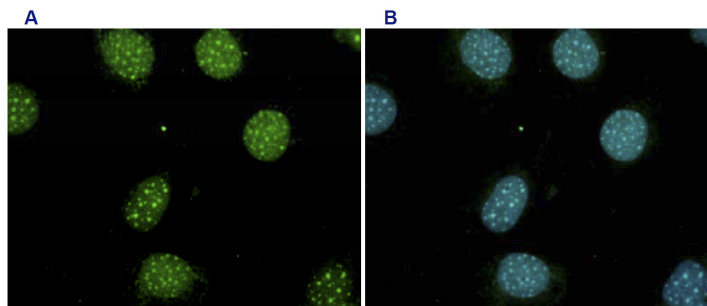
A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K79me3 [Cat. No. CS-068-100] with peptides containing other modifications of histone H3. These include mono- and dimethylation of the same lysine and mono-, di- and trimethylation of lysine 9, 27 and 36. One hundred to 0.2 pmol of the peptides were spotted on a membrane. The antibody was used at a dilution of 1:50,000. Figure 2 shows a high specificity of the antibody for the modification of interest.



**Figure 3**

**Western blot analysis using the Diagenode antibody directed against H3K79me3**

Western blot was performed on histone extracts from HeLa cells (15 µg) with the Diagenode antibody against H3K79me3 [Cat. No. CS-068-100], diluted 1:500 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.



**Figure 4**

**Immunofluorescence using the Diagenode antibody directed against H3K79me3**

Mouse fibroblasts (NIH3T3 cells) were stained with the Diagenode antibody against H3K79me3 [Cat. No. CS-068-100] and with DAPI. Cells were formaldehyde fixed, permeabilized with Triton X100 and blocked with PBS containing 2.5% BSA. Figure 4A: cells were immunofluorescently labeled with the H3K79me3 antibody (diluted 1:200 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to FITC. Figure 1B: staining of the nuclei with DAPI, which specifically labels DNA. Both antibody and DAPI staining are restricted to the nucleus.