



H3K79me3 polyclonal antibody

Cat. No. C15410068

Type: Polyclonal ChIP grade/ChIP-seq grade

Source: Rabbit

Lot #: A246-0040

Size: 50 µg/ 66 µl

Concentration: 0.76 µg/µl

Specificity: Human, mouse, yeast: positive
Other species: not tested

Purity: Affinity purified polyclonal antibody in 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 H3 containing the trimethylated lysine 79 (H3K79me3), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP *	2 µg/ChIP	Fig 1,2
ELISA	1:100 - 1:500	Fig 3
Dot blotting	1:20,000	Fig 4
Western blotting	1:1,000	Fig 5
Immunofluorescence	1:300	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 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.

Results

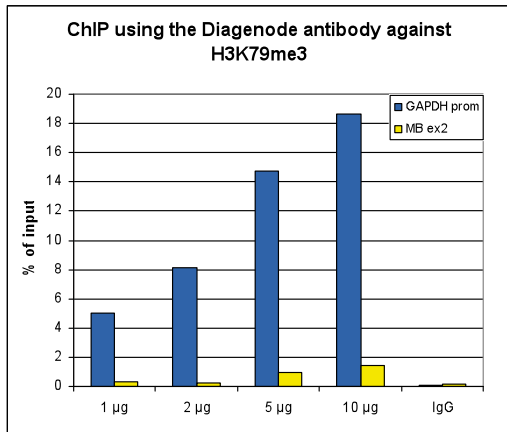


Figure 1. ChIP results obtained with the Diagenode antibody directed against H3K79me3

ChIP was performed with the Diagenode antibody against H3K79me3 (Cat. No. C15410068) on sheared chromatin from 1 million HeLaS3 cells using the “Auto Histone ChIP-seq” kit (Cat. No. C01010022) and the SX-8G IP-Star automated system. A titration of the antibody consisting of 1, 2, 5 and 10 µg 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 (Cat. No. C17011047) and for exon 2 of the inactive myoglobin gene (cat. No. C17011006). Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis). These results are in accordance with the observation that H3K79me3 shows a preference for active promoters.

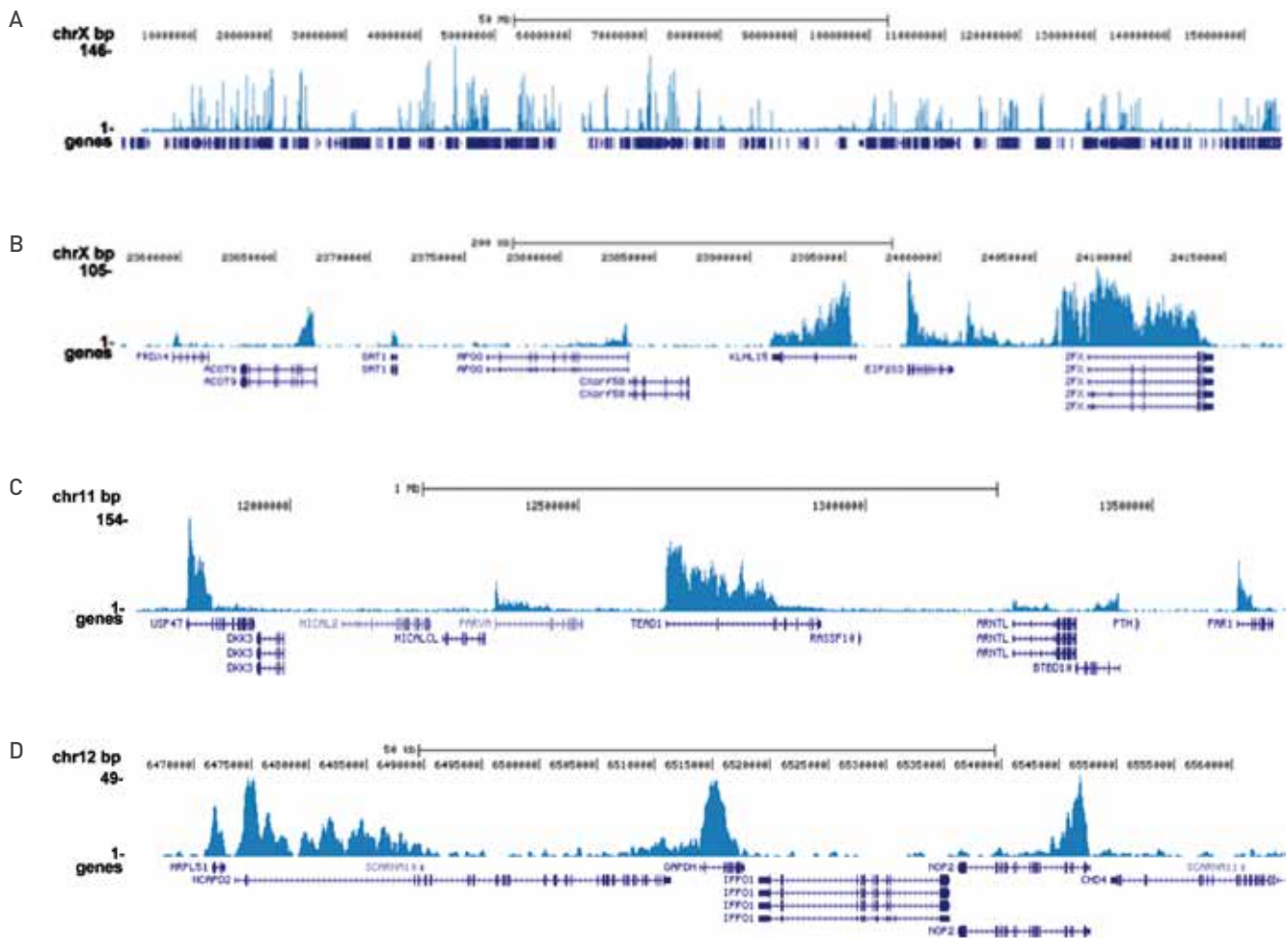


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against H3K79me3

ChIP was performed as described above using 2 µg of the Diagenode antibody against H3K79me3 (Cat. No. C15410068). The IP'd DNA was analysed on an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure 2 shows the peak distribution along the complete sequence and a 600 kb region of the X-chromosome (figure 2A and B), in a 2 Mb region from chromosome 11 (figure 2C), and in a 100 kb region surrounding the GAPDH positive control gene (figure 2D).

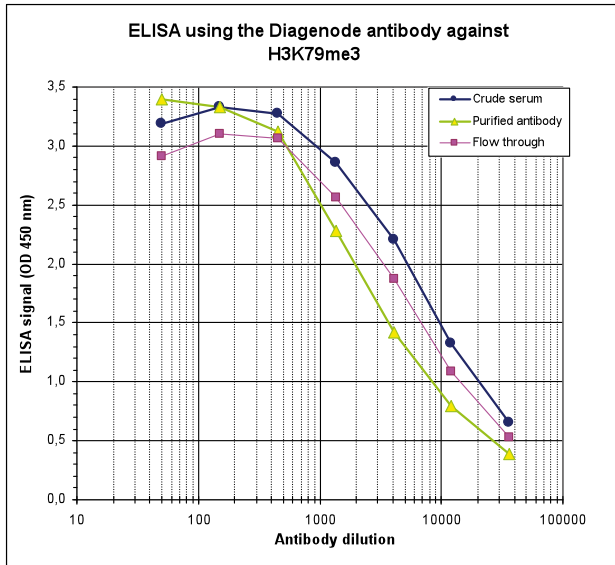


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Diagenode antibody directed against H3K79me3 (Cat. No. C15410068), crude serum and flow through 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 1:3,500.

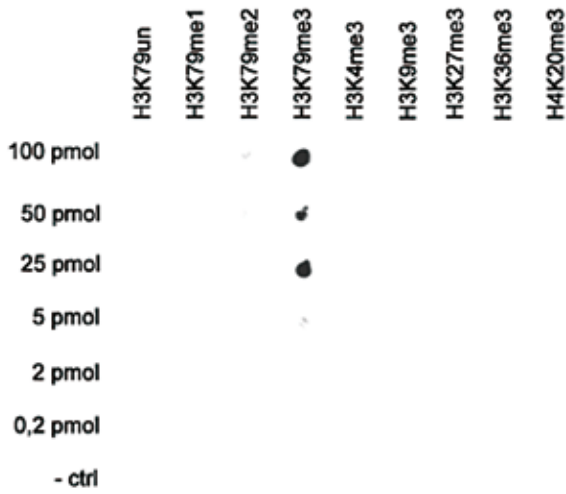


Figure 4. Cross reactivity tests using the Diagenode antibody directed against H3K79me3

A Dot Blot analysis was performed to test the cross reactivity of the Diagenode antibody against H3K79me3 (Cat. No. C15410068) with peptides containing other histone modifications and the unmodified H3K79. One hundred to 0.2 pmol of the respective peptides 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.

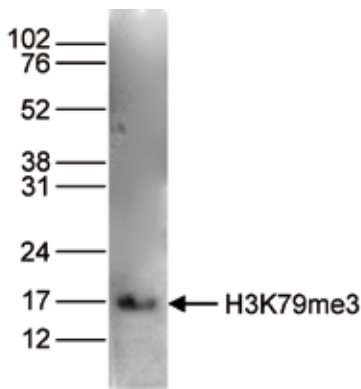


Figure 4. Western blot analysis using the Diagenode antibody directed against H3K79me3

Histone extracts of HeLa cells (15 µg) were analysed by Western blot using the Diagenode antibody against H3K79me3 (Cat. No. C15410068), diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The molecular weight marker is shown on the right; the location of the protein of interest is indicated on the left.

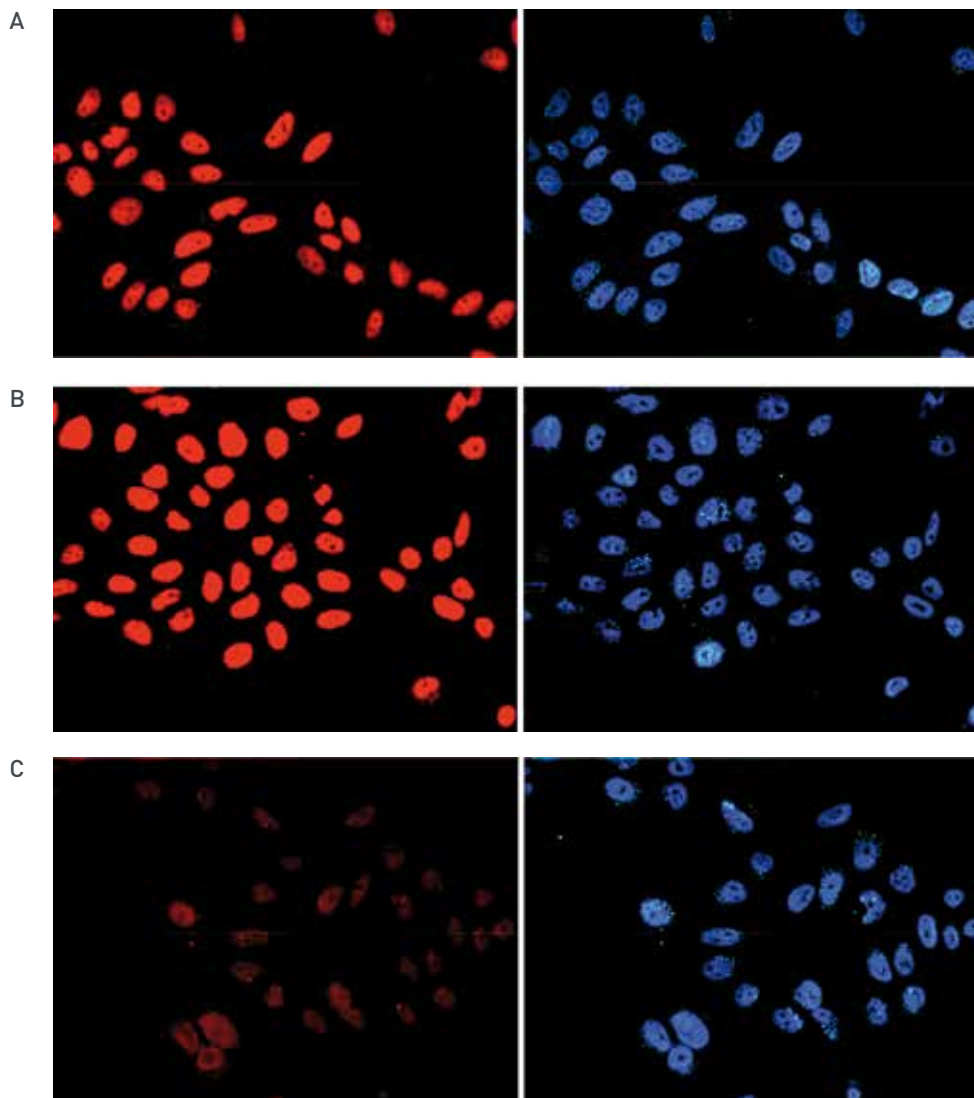


Figure 6. Immunofluorescence using the Diagenode antibody directed against H3K79me3

Human osteosarcoma (U2OS) cells were stained with the Diagenode antibody against H3K79me3 [Cat. No. C15410068] and with DAPI. Cells were fixed with 2.5% formaldehyde for 30' and blocked with PBS/TX-100 containing 1% BSA. Figure 6A: cells were immunofluorescently labeled with the H3K79me3 antibody (left) diluted 1:300 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa568 or with DAPI (right). Figure 6B and C: staining of the cells with the H3K79me3 antibody after incubation of the antibody with 2 ng/ μ l H4K20me3 and H3K79me3 peptide, respectively.

Diagenode sa. BELGIUM | EUROPE

LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode Inc. USA | NORTH AMERICA

400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

Last update: September 30, 2015