diagenode
Innovating Epigenetic Solutions

H2AK119ub polyclonal antibody

Cat. No. C15410002

Type: Polyclonal ChIP-grade

Source: Rabbit

Lot #: A1615-001P

Size: 50 µg/46 µl

Concentration: 1.1 µg/µl

Specificity: Human: 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 the region of histone H2A containing the ubiquitinylated lysine 119 (H2AK119ub), using a KLH-conjugated synthetic peptide.

Applications

	Suggested dilution	Results
ChIP*	2-5 µg per IP	Fig 1
ELISA	1:200	Fig 2
Dot blotting	1:20,000	Fig 3
Western blotting	1:200	Fig 4

*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. Ubiquitinylation of histone H2AK119 is associated with Polycomb mediated gene silencing.

Results

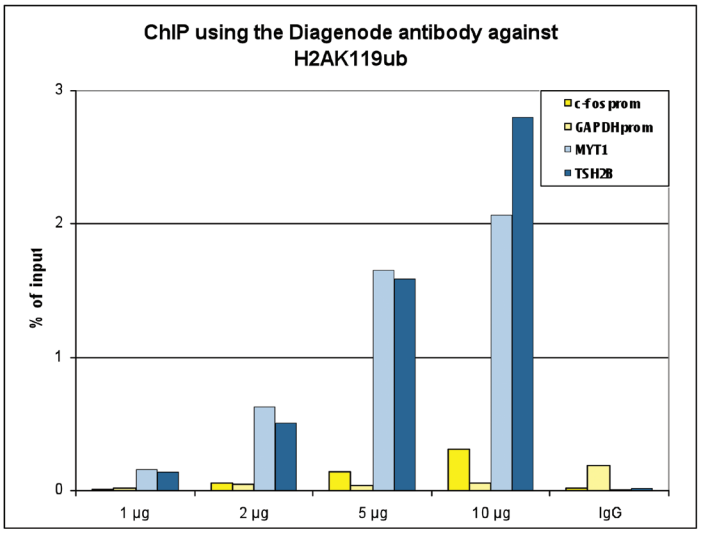

Figure 1. ChIP results obtained with the Diagenode antibody directed against H2AK119ub

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H2AK119ub (cat. No. C15410002) and optimized PCR primer pairs for qPCR. ChIP was performed with the "Auto Histone ChIP-seq" kit (cat. No. AB-Auto02-A100), using sheared chromatin from 1 million cells on the SX-8G IP-Star automated system. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers for the promoters of the active GAPDH and c-fos genes, used as negative controls, and for the inactive MYT1 and TSH2B genes, used as a positive controls.

Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

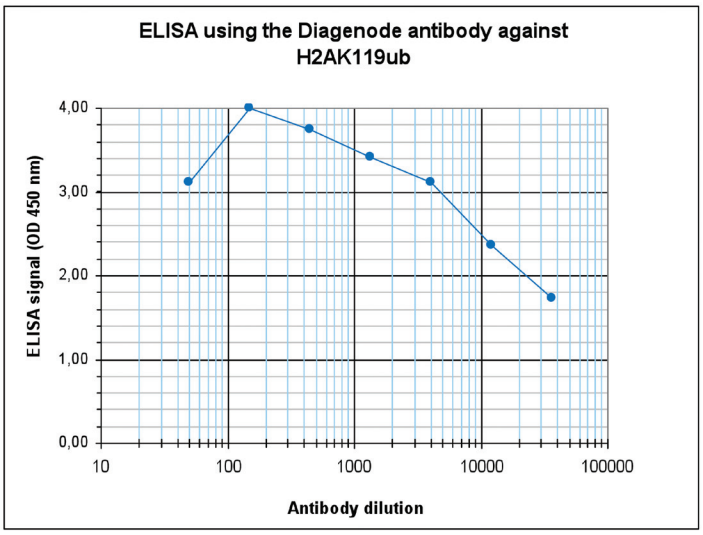

Figure 2. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against H2AK119ub (cat. No. C15410002). 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 1:23,000.

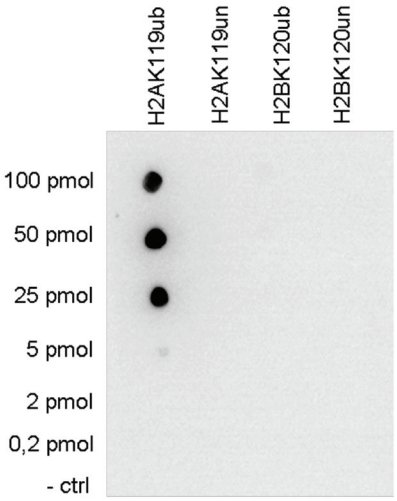

Figure 3. Cross reactivity tests using the Diagenode antibody directed against H2AK119ub

To test the cross reactivity of the Diagenode antibody against H2AK119ub (cat. No. C15410002), a Dot Blot analysis was performed with peptides containing other histone ubiquitinylations and unmodified sequences. 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 3 shows a high specificity of the antibody for the modification of interest.

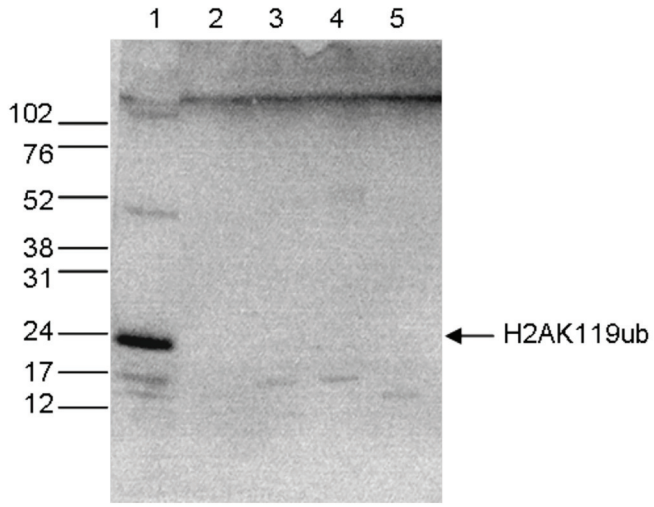

Figure 4. Western blot analysis using the Diagenode antibody directed against H2AK119ub

Western blot was performed on whole cell extracts from HeLa cells (25 µg, lane 1), and on 1 µg of recombinant histone H2A, H2B, H3 and H4 (lane 2, 3, 4 and 5, respectively) using the Diagenode antibody against H2AK119ub (cat. No. C15410002). The antibody was diluted 1:200 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.

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