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Data Sheet

Anti-H2AK119ub polyclonal antibody Catalog #: 25229

Lot #: 230210	Host Species: Rabbit
Conc.: 1.1 mg/ml	Species Reactivity: Human
Size: 50 µg	Immunogen: Synthetic peptide
Clonality: Polyclonal	Purification: Affinity purified

Description: Polyclonal antibody raised in rabbit against the region of histone H2A containing the ubiquitinated lysine 119 (H2AK119ub), using a KLH-conjugated synthetic peptide

Background: 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.

Formulation: PBS containing 0.05% azide and 0.05% ProClin 300

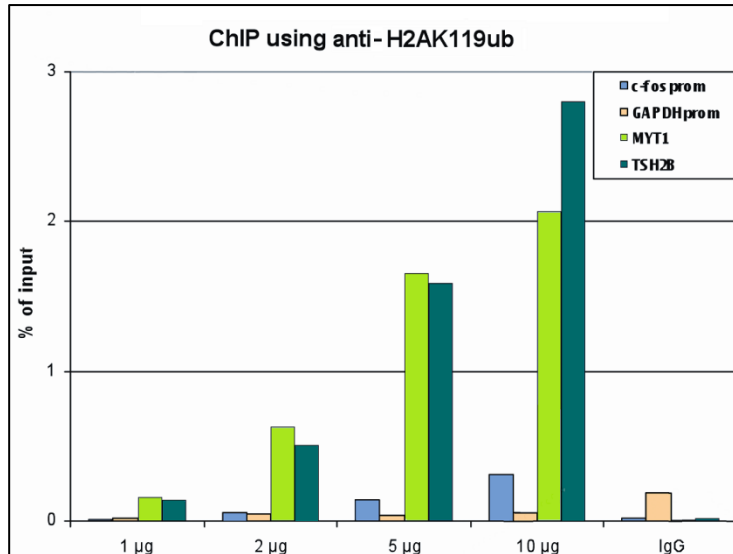
Applications: ChIP (2 - 5 µg/IP), ELISA (1:200), DB (1:20,000), WB (1:200)

Storage/Stability: Store at -80°C for up to 2 years. Centrifuge after first thaw to maximize product recovery. Aliquot to avoid repeated freeze/thaw cycles. Aliquots may be stored at -20°C for at least one month.

Warnings: Avoid freeze/thaw cycles

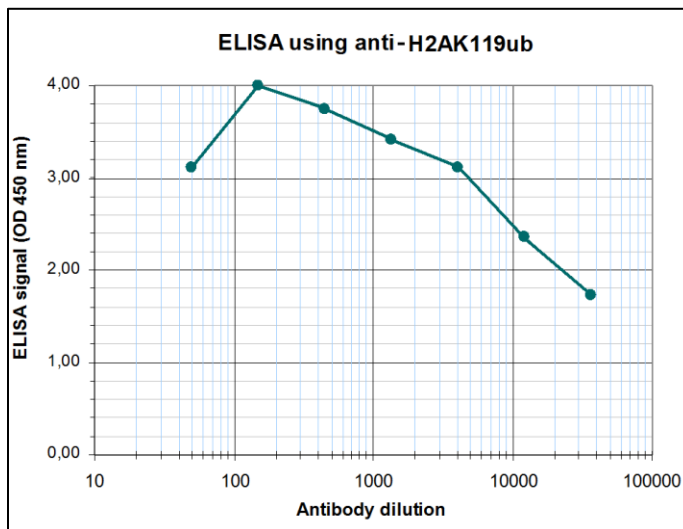
Notes: The optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP

Quality Assurance:



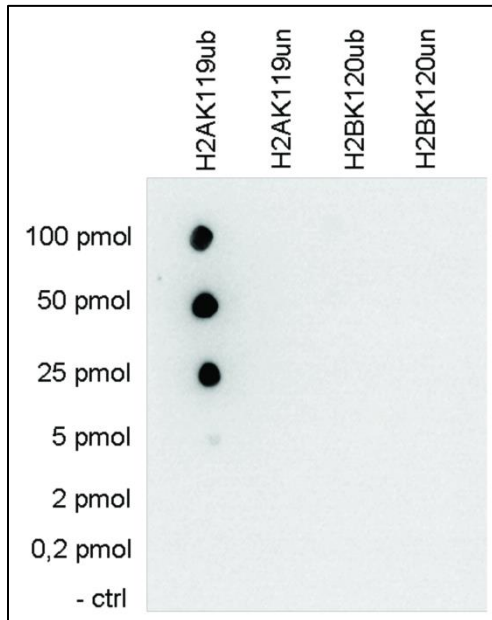
ChIP results obtained with the antibody directed against H2AK119ub.

ChIP assays were performed using human HeLa cells, the antibody against H2AK119ub (cat. No. 25229) and optimized PCR primer pairs for qPCR. ChIP was performed 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).



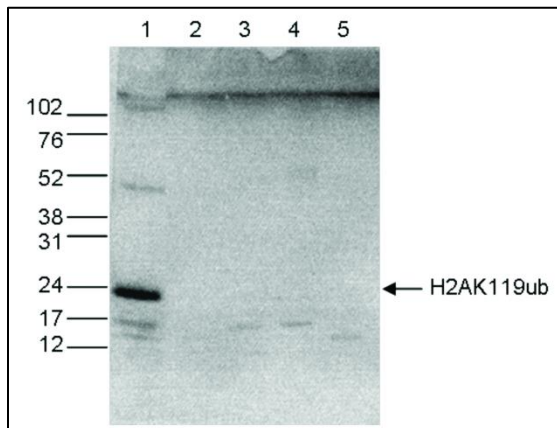
Determination of the antibody titer.

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody against H2AK119ub (cat. No. 25229). 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.



Cross reactivity tests using the antibody directed against H2AK119ub.

To test the cross reactivity of the antibody against H2AK119ub (cat. No. 25229), 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.



Western blot analysis using the 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 antibody against H2AK119ub (cat. No. 25229). The antibody was diluted 1:200 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left.