

yeast H2BK123ub monoclonal antibody

Cat. No. C15200232-50

Type: Monoclonal	Specificity: Human, Yeast
Isotype: NA	Concentration: 1 µg/µl
Source: Mouse	Purity: Affinity purified monoclonal antibody in PBS containing 0.02% azide.
Lot No.: 001	Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Size: 50 µg/ 50 µl	Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description

Monoclonal antibody raised in mouse against the region of yeast histone H2B containing the the ubiquitylated lysine 123 (H2BK123ub), using a KLH-conjugated synthetic peptide. The antibody also recognizes the human homologue H2BK120ub.

Applications

Applications	Suggested dilution	References
ChIP *	2 µg/ChIP	Fig 1, 2, 3
Western Blotting	1:500	Fig 4, 5

* 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. H2B ubiquitination is associated with the ORFs of actively transcribed genes.

Validation data

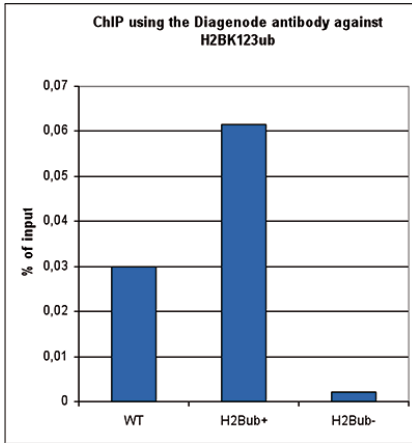


Figure 1. ChIP results obtained with the Diagenode antibody directed against H2BK123ub

ChIP assays were performed using the Diagenode antibody against H2BK123ub (Cat. No. C15200232) on sheared chromatin from WT yeast cells, yeast cells with higher steady state levels of H2BK123ub and yeast cells with no ubiquitinated H2B. Quantitative PCR was performed with primers for the coding region of an active gene. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

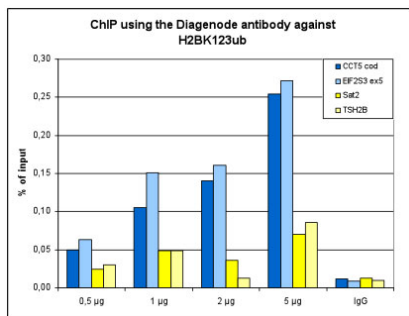


Figure 2. ChIP results obtained with the Diagenode monoclonal antibody directed against H2BK123ub

ChIP assays were performed using human HeLa cells, the Diagenode antibody against H2BK123ub (cat. No. C15200232) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (cat. No. C01010055) on sheared chromatin from 1,000,000 cells. A titration of the antibody consisting of 0.5, 1, 2 and 5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the coding regions of the active CCT5 and EIF2S3 genes, used as positive controls, and for the inactive TSH2B gene and the Sat2 satellite repeat region used as negative controls. Figure 2 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

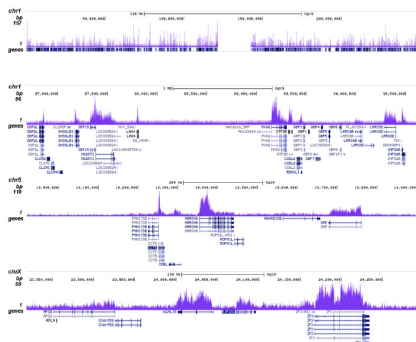


Figure 3. ChIP-seq results obtained with the Diagenode monoclonal antibody directed against H2BK123ub

ChIP was performed with 2 µg of the Diagenode antibody against H2BK123ub (cat. No. C15200232) on sheared chromatin from 1,000,000 HeLa cells using the "iDeal ChIP-seq" kit as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 3 shows the peak distribution along the complete sequence and a 3 Mb region of human chromosome 1 (figure 2A and B) and in two genomic regions surrounding the CCT5 and EIF2S3 positive control genes (figure 2C and D).

Figure 4. Western blot analysis using the Diagenode antibody directed against H2BK123ub

Western blot was performed on histone extracts from HeLa cells (15 µg) using the Diagenode antibody against H2BK123ub (Cat. No. C15200232). The antibody was diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

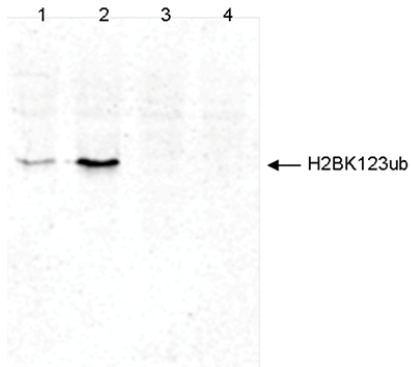
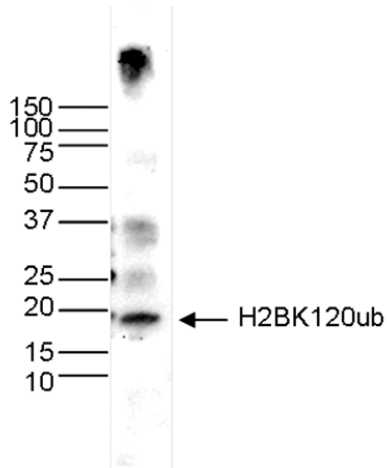


Figure 5. Western blot analysis using the Diagenode antibody directed against H2BK123ub

Western blot was performed on whole cell extracts from WT yeast cells (lane 1), yeast cells with higher steady state levels of H2BK123ub (lane 2), none ubiquitinated H2B (lane 3) and ubiquitinated H2A (lane 4). The position of the protein of interest is indicated on the right.