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Data Sheet

Anti-H3K56ac polyclonal antibody Catalog #: 25259

Lot #:	Host Species: Rabbit
Conc.: 50 µg/ 84 µl	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 H3 containing the acetylated lysine 56 (H3K56ac), using a KLH-conjugated synthetic peptide.

Background:

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

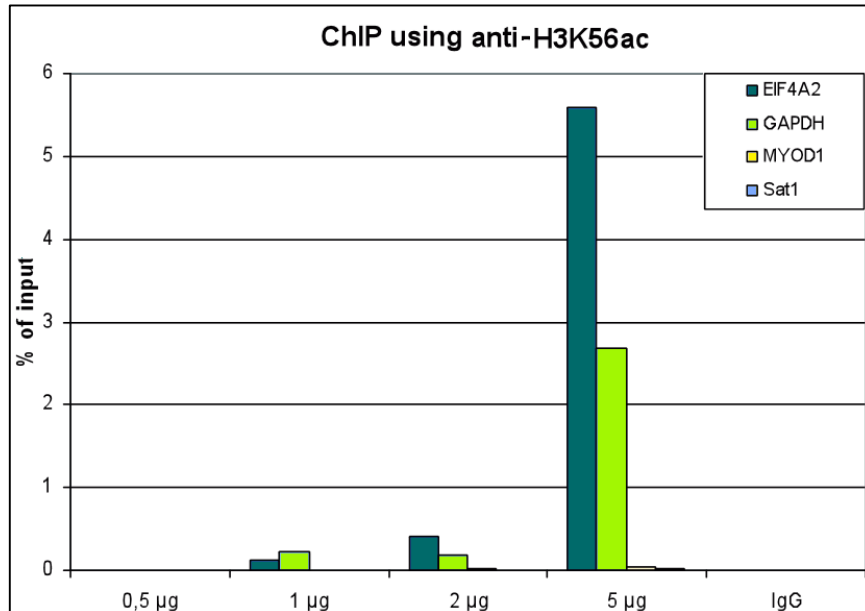
Applications: ChIP/ChIP - seq (5 µg/ChIP), ELISA (1:500), WB (1:20,000)

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

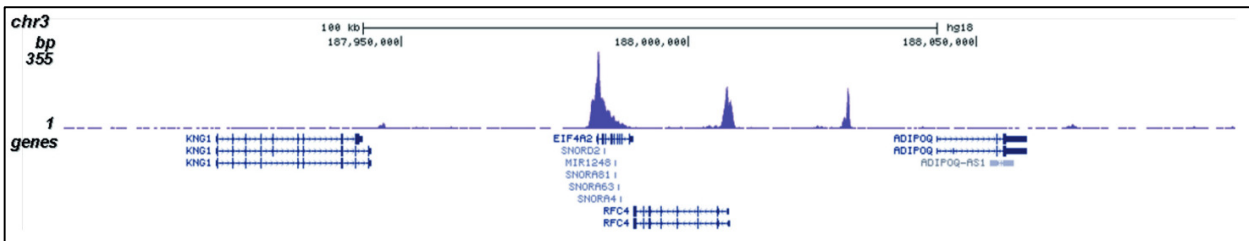
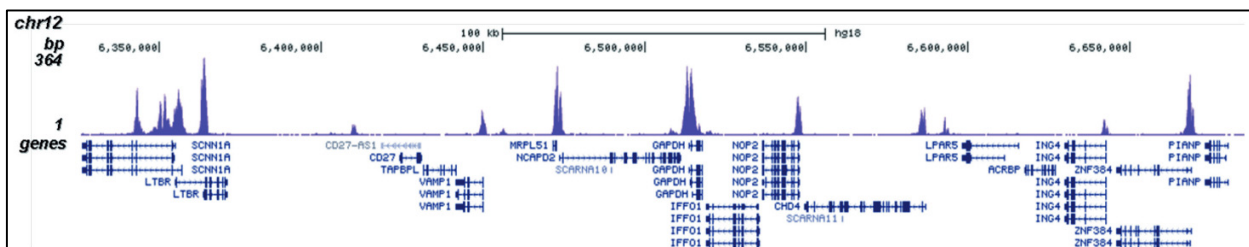
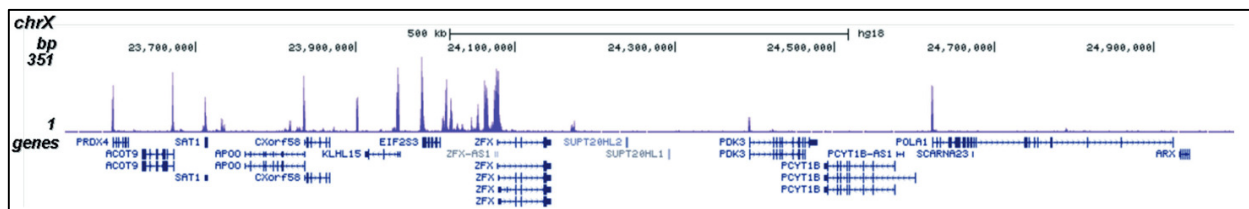
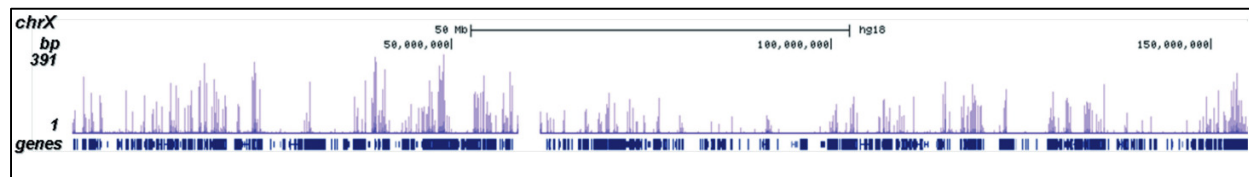
Notes: The optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP

Quality Assurance:



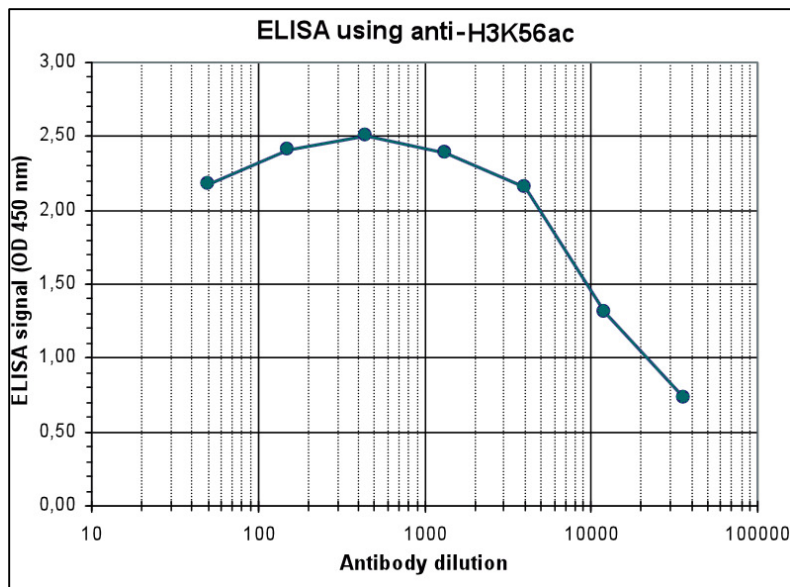
ChIP results obtained with the antibody directed against H3K56ac.

ChIP assays were performed using human HeLa cells, the antibody against H3K56ac (Cat. No. 25259) and optimized PCR primer sets for qPCR. ChIP was performed using sheared chromatin from 1.5 million 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 a region approximately 1 kb upstream of the GAPDH promoter and for the EIF4A2 promoter, used as positive controls, and for the coding region of the inactive MYOD1 gene and the Sat2 satellite repeat, used as negative controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



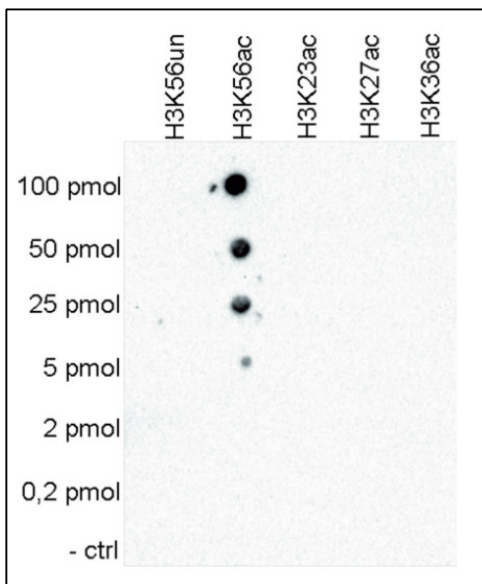
ChIP-seq results obtained with the antibody directed against H3K56ac.

ChIP was performed on sheared chromatin from 1.5 million HeLaS3 cells using 5 µg of the antibody against H3K56ac (cat. No. 25259) as described above. The immunoprecipitated DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment along the complete sequence and a 1 Mb region of the X-chromosome (fig 2A and B) and in genomic regions of chromosome 12 and 3, surrounding the GAPDH and EIF4A2 positive control genes.



Determination of the antibody titer.

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody directed against H3K56ac (Cat. No. 25259) 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 2), the titer of the antibody was estimated to be 1:15,300.



Cross reactivity tests using the antibody directed against H3K56ac.

To test the cross reactivity of the antibody against H3K56ac (Cat. No. 25259), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K56. 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.