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Product no AS16 3190-10

H3K4me3 | Histone H3, trimethylated lysine 4 (H3K4me3) (10 µg)

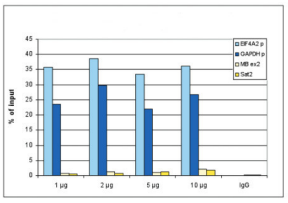
Product information

Immunogen	KLH-conjugated synthetic peptide
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum.
Format	Liquid
Quantity	10 µg
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.

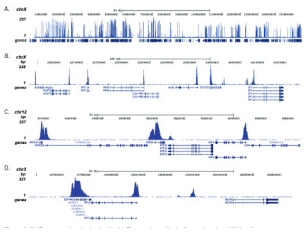
Application information

Recommended dilution	1-5 µg/IP (ChIP-seq), 1 : 10 000 (Dot), 1 : 100 (ELISA), 1 : 200 (IF), 1 : 2000 (PA), 1 : 1000 (WB)
Confirmed reactivity	Human
Predicted reactivity	*Arabidopsis thaliana, Solanum lycopersicum, Oryza sativa,Populus sp., Zea mays*
Not reactive in	No confirmed exceptions from predicted reactivity are currently known

application example



ChIP assays were performed using human HeLa cells, the antibody against H3K4me3 and optimized PCR primer pairs for qPCR. ChIP was performed with the “Auto Histone ChIP-seq” kit, using sheared chromatin from 1 million cells. 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 specific for the promoter of the active genes GAPDH and EIF4A2, used as positive controls, and for exon 2 of the inactive myoglobin (MB) 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). These results are in accordance with the observation that trimethylation of K4 at histone H3 is associated with the promoters of active genes.



ChIP-seq was performed on sheared chromatin from 1 million HeLaS3 cells using 1 µg of the anti-H3K4me3 antibodies as described above. The IP’d DNA was subsequently 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. Image shows the peak distribution along the complete sequence and a 600 kb region of the X-chromosome (A and B) and in two regions surrounding the GAPDH and EIF4A2 positive control genes, respectively (C and D). These results clearly show an enrichment of the H3K4 trimethylation at the promoters of active genes.

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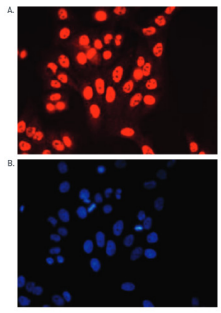
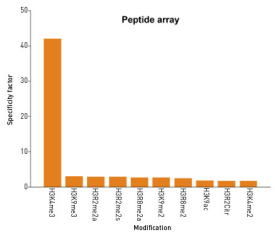
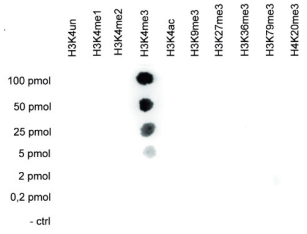


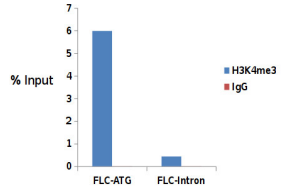
Immunofluorescence: human osteosarcoma (U2OS) cells were stained using anti-H3K4me3 antibodies and with DAPI. Cells were fixed with 4% formaldehyde for 20' and blocked with PBS/TX-100 containing 5% normal goat serum. The cells were immunofluorescently labeled with the H3K4me3 antibody (left) diluted 1:200 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa568 or with DAPI (right), which specifically labels DNA.



Peptide array: the specificity of this antibody was demonstrated by peptide array analyses on an array containing 384 peptides with different combinations of modifications from histone H3, H4, H2A and H2B. The antibody was used at a dilution of 1:2 000.This figure shows a high specificity for the peptides containing the H3K4me3 modification.



Dot blot: to test the cross reactivity of anti-H3K4me3 antibodies, a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K4. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:10 000. This figure shows a high specificity of the antibody for modification of interest.



ChIP: *Arabidopsis thaliana* (Col-0) seedlings 14 days after germination using our premium anti-H3K4me3 ChIP-seq grade antibody and rabbit IgG. Error bars represent the standard deviation of at least 3 biological repetitions. 1 µg of H3K4me3 antibody and 1 µg of the negative IgG control were used per ChIP reaction. Quantitative PCR was performed with the positive control FLC-ATG and the negative control FLC-Intron1 primer sets from the kit. The recovery is expressed as % of input.