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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

Anti-H3K9me3 polyclonal antibody

Catalog #: 25272

Lot #: 220420	**Host Species:** Rabbit
Conc.: 1.85 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 H3 containing the trimethylated lysine 9 (H3K9me3), using a KLH-conjugated synthetic peptide.

Background: Trimethylation of histone H3K9 is associated with satellite repeat regions and ZNF repeat genes.

Formulation: PBS containing 0.05% azide.

Applications: ChIP/ChIP - seq (0.5 - 1 µg/ChIP), ELISA (1:1000 - 1:10,000), DB (1:2000), WB (1:2000)

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 0.5-5 µg per IP

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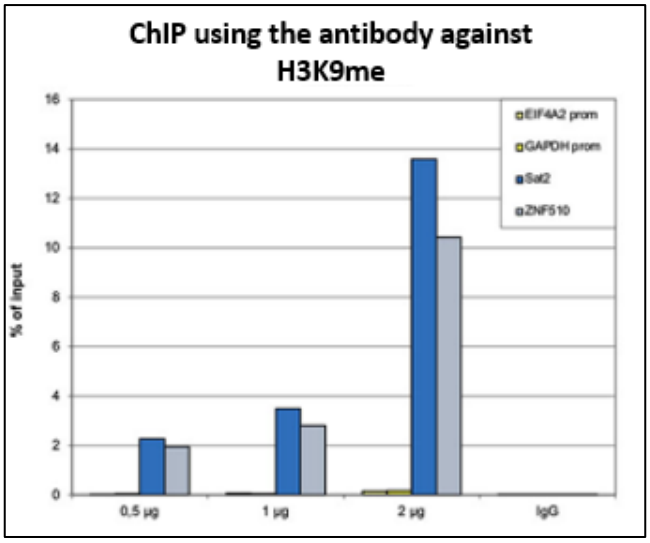
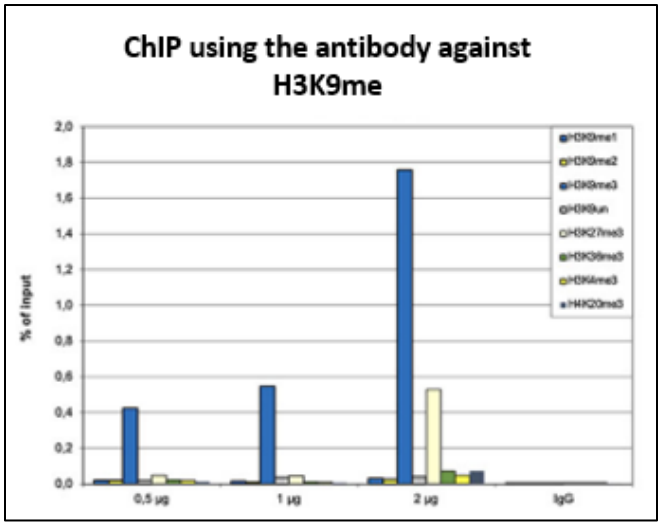
Quality Assurance:





ChIP results obtained with the antibody directed against H3K9me3

ChIP was performed with the antibody against H3K9me3 (BPS Bioscience #25272) on sheared chromatin from 500,000 K562 cells. The chromatin was spiked with a panel of in vitro assembled nucleosomes, each containing a specific lysine methylation. A titration of the antibody consisting of 0.5, 1 and 2 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control.

Figure 1A. Quantitative PCR was performed with primers for the ZNF510 gene and the Sat2 satellite repeat, used as positive controls, and for the EIF4A2 and GAPDH promoters, used as negative controls. The graph shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

Figure 1B. Recovery of the nucleosomes carrying the H3K9me1, H3K9me2, H3K9me3, H3K4me3, H3K27me3, H3K36me3, H4K20me3 modifications and the unmodified H3K9 as determined by qPCR. The figure clearly shows the antibody is very specific in ChIP for the H3K9me3 modification when using 0.5 or 1 µg. With 2 µg of antibody, some recovery of the H3K27me3 nucleosome is observed.

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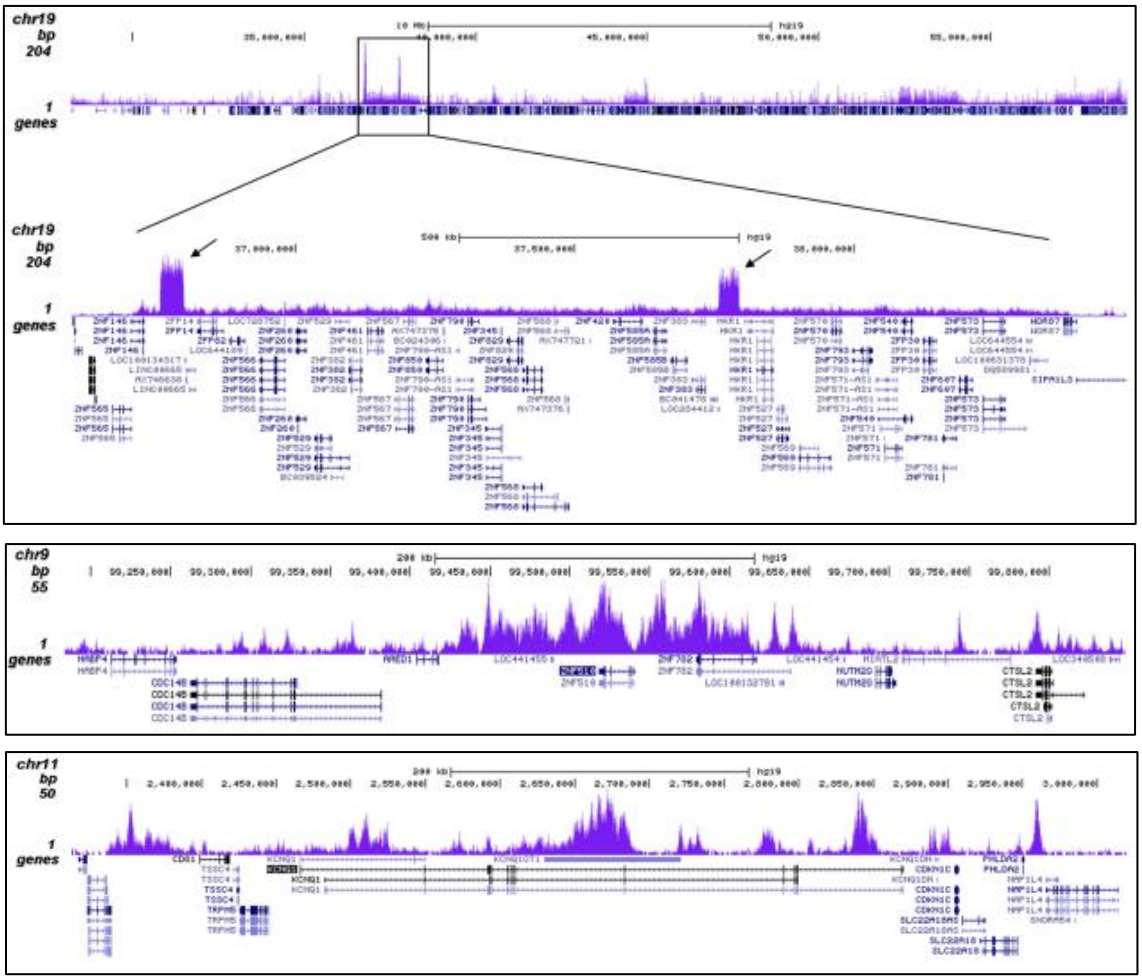

ChIP-seq results obtained with the antibody directed against H3K9me3.
ChIP was performed with 1 µg of the antibody against H3K9me3 (BPS Bioscience #25272) on sheared chromatin from 500,000 K562 cells. The IP'd DNA was subsequently analysed on an Illumina HiSeq4000. Library preparation, cluster generation 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 2A shows the signal distribution along the long arm of chromosome 19 and a zoom-in to an enriched region containing several ZNF repeat genes. The arrows indicate two satellite repeat regions which exhibit a stronger signal. Figures 2B and 2C show the enrichment along the ZNF510 positive control target and at the KCNQ1 imprinted gene.

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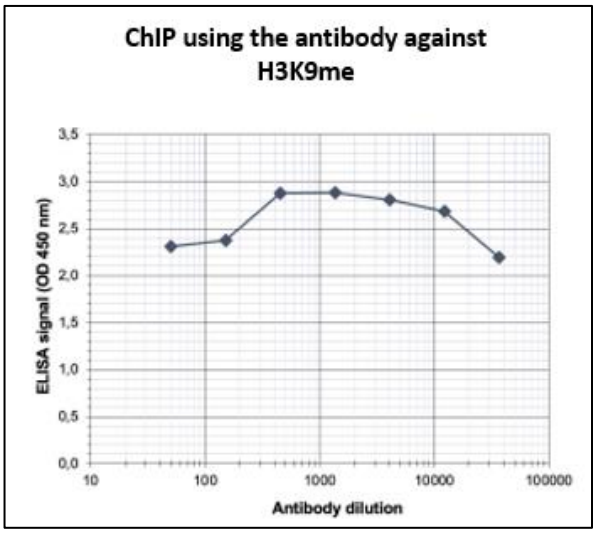
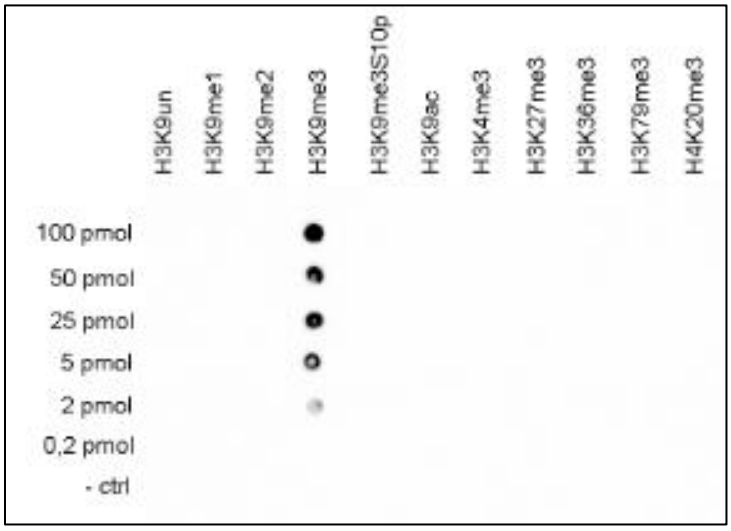


Determination of the antibody titer.

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the antibody directed against human H3K9me3 (BPS Bioscience #25272) 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 3), the titer of the antibody was estimated to be 1:198,000.



Cross reactivity tests using the antibody directed against H3K9me3.

A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K9me3 (BPS Bioscience #25272) with peptides containing other modifications of histone H3 and H4 and the unmodified sequence of histone H3. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:2,000. Figure 4 shows a high specificity of the antibody for the modification of interest.

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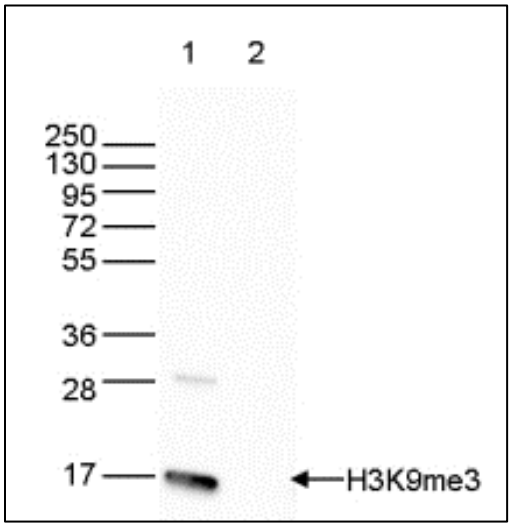

Western blot analysis using the antibody directed against H3K9me3.
Western blot was performed on 40 µg whole cell extracts from HeLa cells (lane 1) and on 1 µg of recombinant histone H3 (lane 2) using the antibody against H3K9me3 (BPS Bioscience #25272). The antibody was diluted 1:2,000 in TBS-Tween 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.