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Produktinformation

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- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

Anti-H3K4me2 polyclonal antibody

Catalog #: 25255

Lot #:	**Host Species:** Rabbit
Conc.: 50 µg/42 µl	**Species Reactivity:** Human
Size: 50 µg	**Immunogen:** Synthetic peptide
Clonality: Polyclonal	**Purification:** Affinity purified

Description: Polyclonal antibody raised in rabbit against histone H3 containing the dimethylated lysine 4 (H3K4me2), using a KLH-conjugated syntheticpeptide

Background:

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

Applications: ChIP/ChIP - seq (5 µg/ChIP), ELISA (1:500), DB (1:20,000), WB (1:1000), IF (1:5000)

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

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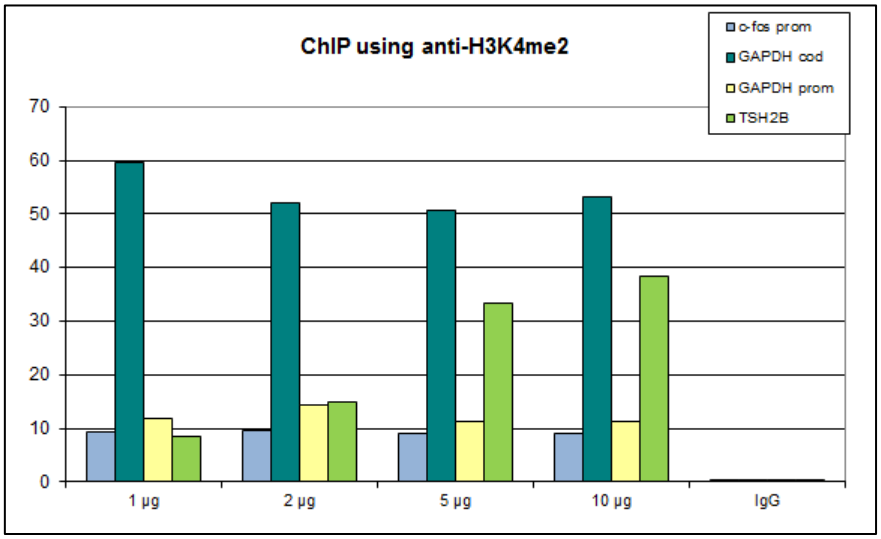
Quality Assurance:



ChIP results obtained with the antibody directed against H3K4me2.
ChIP was performed with the antibody against H3K4me2 (Cat. # 25255) on sheared chromatin from 1 million HeLaS3 cells. A titration of the antibody consisting of 1, 2, 5 and 10 µg per ChIP experiment was analyzed. IgG (2 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for the promoter and coding region of the active GAPDH gene, the promoter of the active c-fos gene and for the coding region of the inactive TSH2B. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

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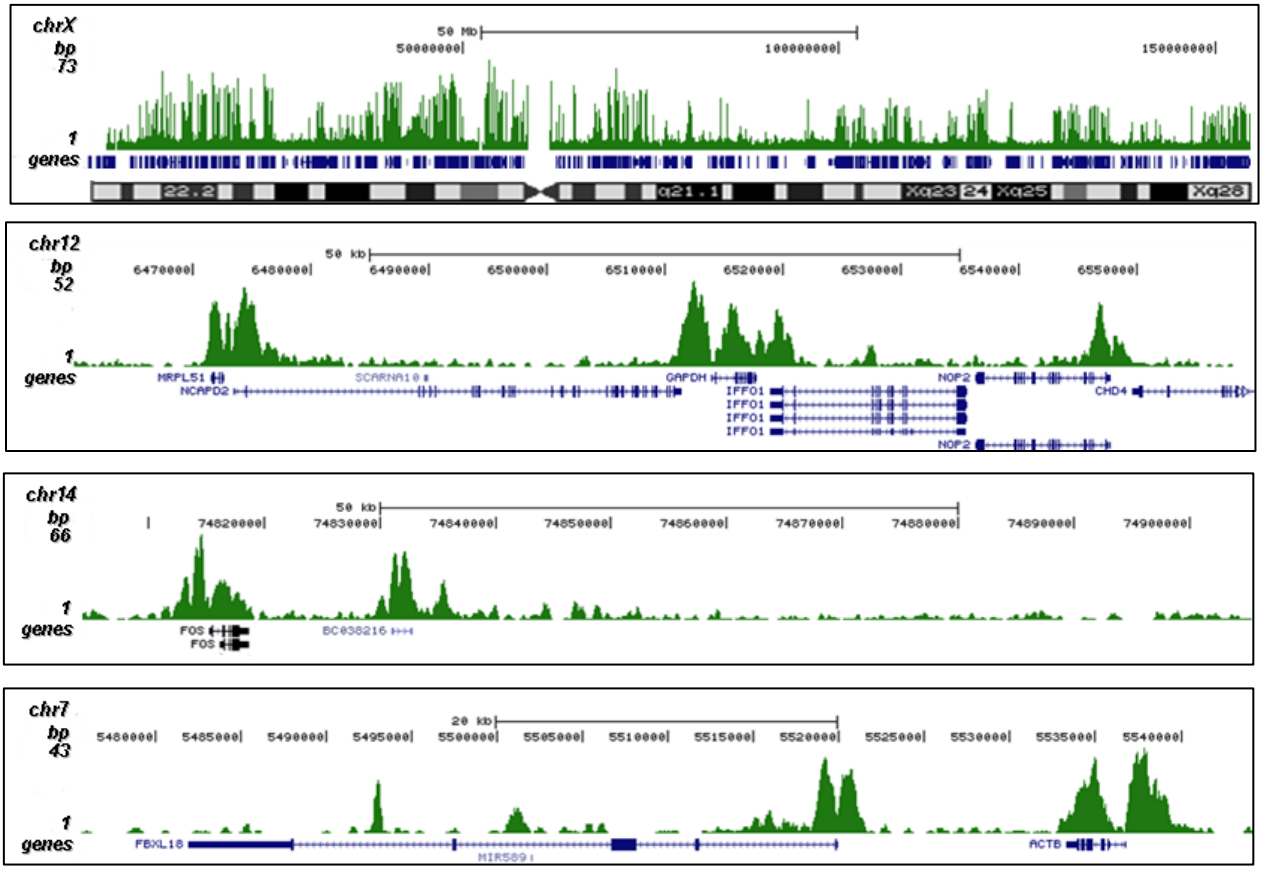

ChIP-seq results obtained with the antibody directed against H3K4me2.
ChIP was performed as described above using 1 µg of the antibody against H3K4me2 (Cat. # 25255). The immunoprecipitated DNA was analyzed on an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure 2 shows the peak distribution along the complete X-chromosome (figure 2A) and in 3 chromosomal regions surrounding the GAPDH, c-fos and ACTB genes (figure 2B, C and D, respectively).

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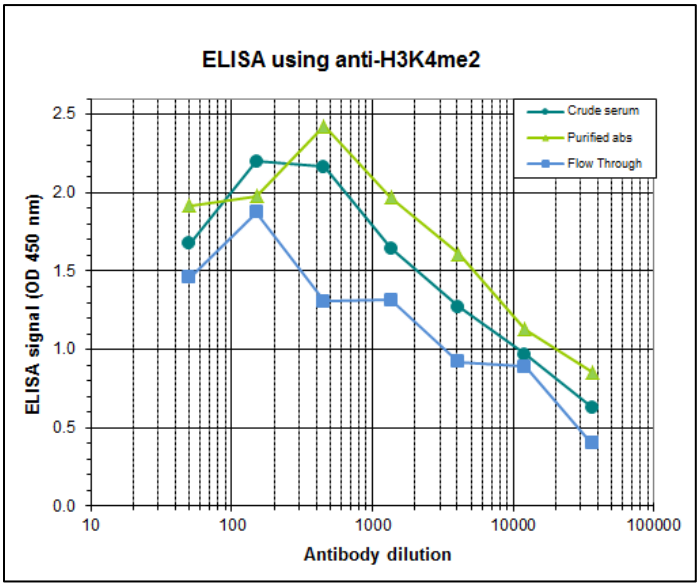
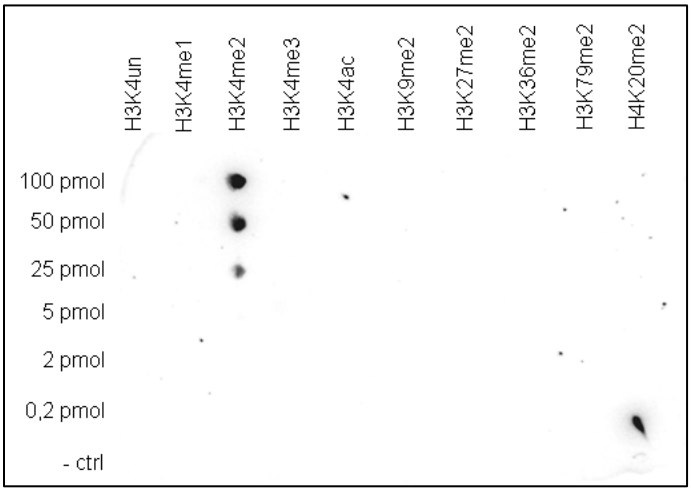
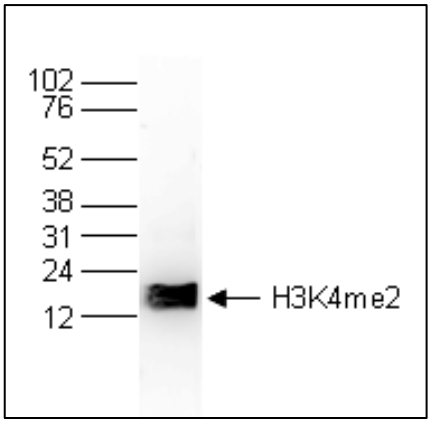


Determination of the titer.
To determine the titer, an ELISA was performed using a serial dilution of the antibody directed against H3K4me2 (Cat. # 25255), crude serum and flow-through in antigen coated wells. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:12,600.



Cross reactivity test using the antibody directed against H3K4me2.
A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K4me2 (Cat. # 25255) with peptides containing other modifications of histone H3 and H4 and the unmodified H3K4 sequence. 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 4 shows a high specificity of the antibody for the modification of interest.



Western blot analysis using the antibody directed against H3K4me2.
Histone extracts of HeLa cells (15 µg) were analyzed by Western blot using the antibody against H3K4me2 (Cat. # 25255) diluted 1:1,000 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.

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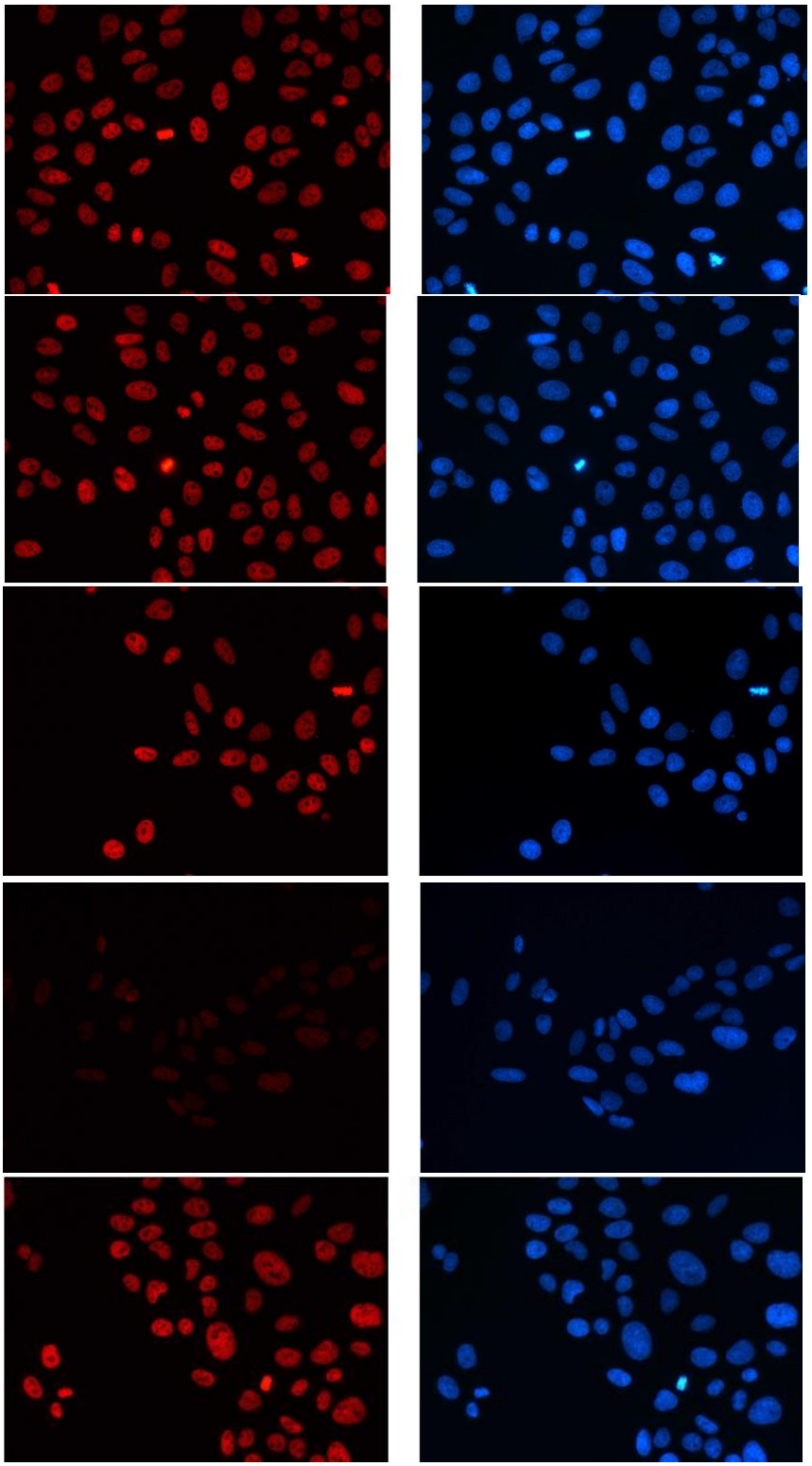

Immunofluorescence using the antibody directed against H3K4me2.
Human osteosarcoma (U2OS) cells were stained with the antibody against H3K4me2 (Cat. # 25255) and with DAPI. Cells were fixed with 4% formaldehyde for 20 minutes and blocked with PBS/TX-100 containing 5% normal goat serum. Figure 6A: cells were immunofluorescently labeled with the H3K4me2 antibody (left) diluted 1:5,000 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa568 or with DAPI (right), which specifically labels DNA. Figure 6B, C, D and E: staining of the cells with the H3K4me2 antibody after incubation of the antibody with 5 ng/µl blocking peptide containing the unmodified and the mono-, di- and trimethylated H3K4, respectively.