6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Anti-H3K4me2 monoclonal antibody

Catalog #: 25254

Lot #:	**Host Species:** Mouse
Conc.: 50 µg/50 µl	**Species Reactivity:** Human
Size: 50 µg	**Immunogen:** Synthetic peptide
Clonality: Monoclonal	**Purification:** Protein A purified

Description: Monoclonal antibody raised in Mouse against histone H3, dimethylated at lysine 4 (H3K4me2), using a KLH-conjugated syntheticpeptide

Background:

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

Applications: ChIP (1 µg/ChIP), ELISA (1:5000), WB (1:1000), IF (1:500)

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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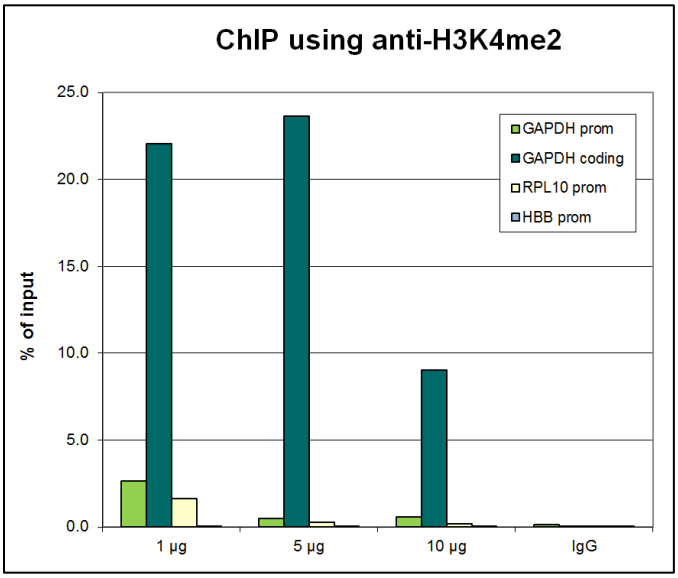
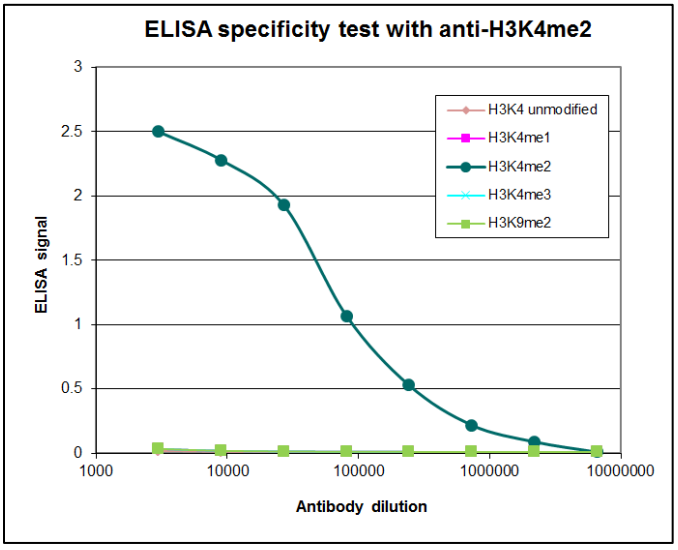
Quality Assurance:



ChIP results obtained with the monoclonal antibody directed against H3K4me2.
ChIP assays were performed using HeLa cells, the monoclonal antibody against H3K4me2 (cat. No. 25254) and optimized PCR primer sets for qPCR. ChIP was performed using sheared chromatin from 1.6 million cells. A titration of the antibody consisting of 1, 5 and 10 µg per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control. QPCR was performed with primers for the promoter and the coding region of the GAPDH gene, and for the RPL10 and HBB promoters. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



Cross reactivity of the monoclonal antibody directed against H3K4me2.
To test the specificity an ELISA was performed using a serial dilution of the monoclonal antibody against H3K4me2 (cat. No. 25254). The wells were coated with peptides containing the unmodified H3K4 as well as the mono-, di- and trimethylated H3K4 and the dimethylated H3K9. Figure 2 shows a high specificity of the antibody for the modification of interest.

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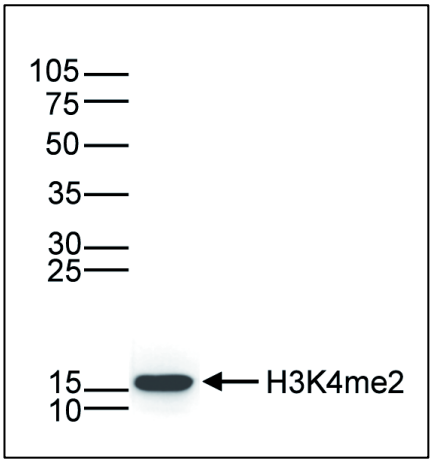
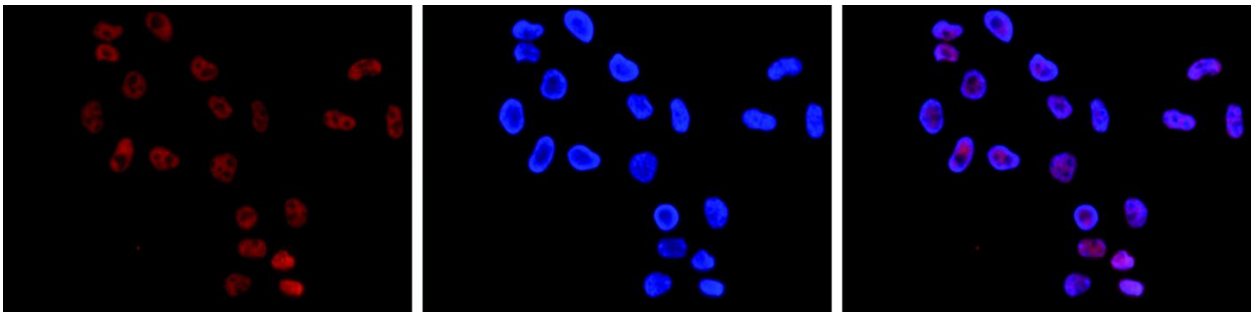


Western blot analysis using the monoclonal antibody directed against H3K4me2.
Histone extracts (15 µg) from HeLa cells were analysed by Western blot using the monoclonal antibody against H3K4me2 (cat. No. 25254) diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



Immunofluorescence using the monoclonal antibody directed against H3K4me2.
HeLa cells were stained with the antibody against H3K4me2 (cat. No. 25254) and with DAPI. Cells were fixed with 4% formaldehyde for 10 minutes and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K4me2 antibody (left) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.