Histone H2BK5ac antibody (pAb)

Catalog Nos: 39123, 39124
RRID: AB_2615079
Isotype: Serum
Application(s): ChIP, ChIP-Seq, DB, IF, WB
Reactivity: Human, Wide Range Predicted

Volumes: 200 µl, 10 µl
Purification: None
Host: Rabbit
Molecular Weight: 15 kDa

Background: Histone H2B is one of the core components of the nucleosome. The nucleosome is the smallest subunit of chromatin and consists of 147 base pairs of DNA wrapped around an octamer of core histone proteins (two each of Histone H2A, Histone H2B, Histone H3 and Histone H4). Histone H1 is a linker histone, present at the interface between the nucleosome core and DNA entry/exit points; it is responsible for establishing higher-order chromatin structure. Chromatin is subject to a variety of chemical modifications, including post-translational modifications of the histone proteins and the methylation of cytosine residues in the DNA. Reported histone modifications include acetylation, methylation, phosphorylation, ubiquitylation, glycosylation, ADP-ribosylation, carbonylation and SUMOylation; they play a major role in regulating gene expression.

Lysine N-ε-acetylation is a dynamic, reversible and tightly regulated protein and histone modification that plays a major role in chromatin remodeling and in the regulation of gene expression in various cellular functions. Histone H2A and Histone H2B are acetylated in bulk chromatin by p300 and form acetylated Histone H2A/Histone H2B heterodimers. When DNA associates with intact core histone octamers that contain acetylated H2A/H2B dimers, the inhibition of transcriptional initiation significantly decreases, indicating that acetylation of their lysine residues may mediate transcription.

Immunogen: This Histone H2B acetyl Lys5 antibody was raised against a peptide including acetyl-lysine 5 of human histone H2B.

Buffer: Rabbit serum containing 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

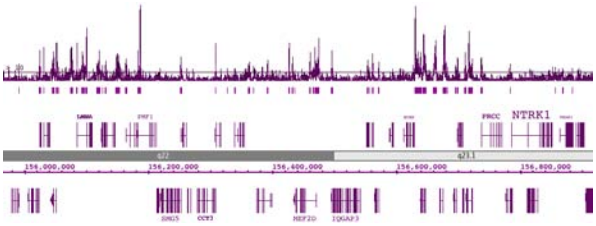
Application Notes:
Applications Validated by Active Motif:
ChIP: 5 - 10 µl per ChIP
WB*: 1:2,000 - 1:10,000 dilution
IF: 1:500 dilution

The modENCODE and NIH Roadmap Epigenomics Mapping Consortiums have implemented rigorous standardization criteria for all assays and reagents to be used. As part of this initiative, antibody specificity testing and the ability of the antibodies to work in ChIP-Seq were assessed in a large-scale study. This Histone H2B acetyl Lys5 antibody was validated for ChIP-Seq in the study (see reference).

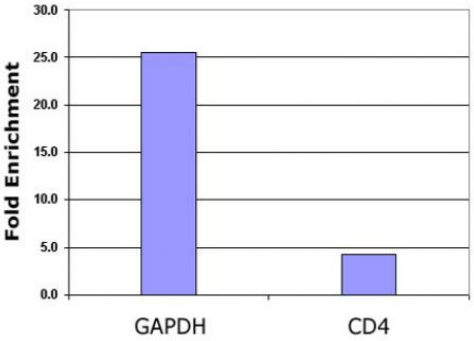
*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western blot.

Storage and Guarantee: Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product is guaranteed for 12 months from date of receipt.

This product is for research use only and is not for use in diagnostic procedures.

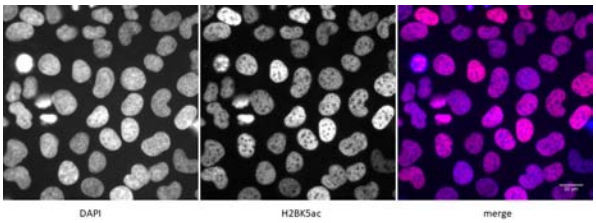


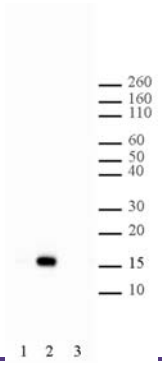
Histone H2B acetyl Lys5 pAb tested by ChIP-Seq.
ChIP was performed using chromatin from the H1 human embryonic stem cell line. ChIP DNA was sequenced on the Illumina GA II and sequence tags were mapped to identify H2BK5Ac binding. The image shows H2BK5Ac binding across a 1 MB region on chromosome 1.



Histone H2B acetyl Lys5 pAb tested by ChIP analysis.
Chromatin IP performed using the ChIP-IT® Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 10^6 cell equivalents per ChIP) using 10 µl of Histone H2B acetyl Lys5 pAb or the equivalent amount of rabbit IgG as a negative control. Real time, quantitative PCR (RT-qPCR) was performed on DNA purified from each of the ChIP reactions using a primer pair specific for the indicated gene. Data are presented as Fold Enrichment of the ChIP antibody signal versus the negative control IgG using the ddCT method.

Detection of H2BK5ac by immunofluorescence.
U2OS cells were stained with H2BK5ac antibody at a dilution of 1:500. Left panel: DAPI. Middle panel: H2BK5ac antibody staining. Right panel: merge.





Histone H2B acetyl Lys5 pAb tested by Western blot.
HeLa acid extract (5 µg per lane) was probed with Histone H2B acetyl Lys5 polyclonal antibody (1:2,000 dilution).
Lane 1: No treatment.
Lane 2: Cells treated with sodium butyrate.
Lane 3: Human recombinant H2B (200 ng).

Histone H2B acetyl Lys5 pAb tested by dot blot analysis.
Dot blot analysis was used to confirm the specificity of Histone H2B acetyl Lys5 pAb for acetyl-Lys5 of histone H2B. Decreasing amounts of modified and unmodified peptides were spotted onto PVDF and probed with the antibody at a dilution of 1:5,000.
Lane 1: Peptide acetylated at lysine 5 of H2B.
Lane 2: Unmodified lysine 5 peptide.
Lane 3: Peptide acetylated at lysine 16 of H2B.
Lane 4: Unmodified lysine 16 peptide.
Lane 5: Peptide acetylated at lysine 120 of H2B.
Lane 6: Unmodified lysine 120 peptide.