

AbFlex® Histone H3.3 antibody (rAb)

Catalog Nos: 91191, 91192

RRID: AB_2793796

Isotype: IgG2a

Application(s): ChIP-Seq, WB

Reactivity: Human

Quantities: 100 µg, 10 µg

Purification: Protein A Chromatography

Host: Mouse

Concentration: 1 µg/µl

Molecular Weight: 17 kDa

Background: AbFlex® antibodies are recombinant antibodies (rAbs) that have been generated using defined DNA sequences to produce highly specific, reproducible antibodies. Each AbFlex antibody contains a 6xHis Tag, a Biotinylation Tag for enzymatic biotin conjugation using the biotin ligase, BirA, and a sortase recognition motif (LPXTG) to attach a variety of labels directly to the antibody including fluorophores, enzymatic substrates (HRP, AP), peptides, drugs as well as solid supports.

AbFlex® Histone H3.3 antibody was expressed as full-length IgG with mouse immunoglobulin heavy and light chains (IgG2a isotype) in mammalian 293 cells.

Histone H3.1 and Histone H3.3 are the two main Histone H3 variants found in plants and animals. They are known to be important for gene regulation. Histone H3.1 and H3.3 have been shown to demonstrate unique genomic localization patterns thought to be associated with their specific functions in regulation of gene activity. Specifically, Histone H3.1 localization is found to coincide with genomic regions containing chromatin repressive marks (H3K9me3, H3K27me3 and DNA methylation), whereas Histone H3.3 primarily colocalizes with marks associated with gene activation (H3K4me3, H2BK120ub1, and RNA pol II occupancy). Deposition of the Histone H3.1 variant into the nucleosome correlates with the canonical DNA synthesis-dependent deposition pathway, whereas Histone H3.3 primarily serves as the replacement Histone H3 variant outside of S-phase, such as during gene transcription. Aberrant localization of these variants is also known to correlate with certain cancers.

Immunogen: This antibody was raised against a peptide corresponding to amino acids 21-39 of human Histone H3.3.

Buffer: Purified IgG in 140 mM Hepes, pH 7.5, 70 mM NaCl, 32 mM NaOAc, 0.035% sodium azide, 30% glycerol. Sodium azide is highly toxic.

Application Notes:

Applications Validated by Active Motif:

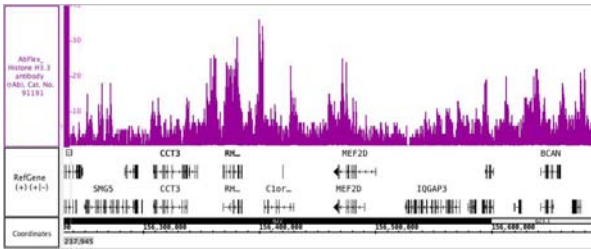
ChIP-Seq: 4 µg per ChIP

WB*: 2 µg/ml dilution

*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.

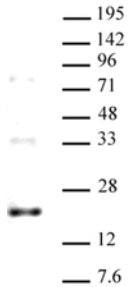
Storage and Guarantee: Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product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

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



ChIP-Seq of AbFlex Histone H3.3 antibody (rAb)

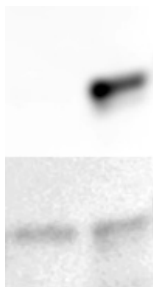
ChIP was performed using the ChIP-IT High Sensitivity Kit (Cat. No. 53040) with 30 µg of chromatin from brain tumor cell cells and 4 µg of AbFlex Histone H3.3 antibody. ChIP DNA was sequenced on the Illumina HiSeq and 15.1 million sequence tags were mapped to identify Histone H3.3 binding sites. The image shows binding across a region of chromosome 1.



AbFlex[®] Histone H3.3 antibody (rAb) tested by Western blot.

20 µg of HeLa cell nuclear extract was run on an SDS-PAGE gel and probed with 2 µg/ml AbFlex Histone H3.3 antibody.

MW: 17 kDa



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AbFlex[®] Histone H3.3 antibody (rAb) tested by Western Blot

Lane 1: 250 ng of Recombinant Histone H3.1 (Cat. #31294); Lane 2: 250 ng of Recombinant Histone H3.3 (Cat. # 31295). Proteins were run on SDS-PAGE, transferred to nitrocellulose and stained with Ponceau S (lower panel) or probed with AbFlex Histone H3.3 antibody (upper panel). The AbFlex antibody only detects, and is specific for, Histone H3.3 as seen by a band at 17 kDa.

For optimal results, primary antibody incubations should be performed at room temperature. The addition of 0.1% Tween 20 to all Blotto solutions may also reduce background. Individual optimization may be required.