

AbFlex® RNA pol II CTD phospho Ser5 antibody (rAb)

Catalog Nos: 91119, 91120

RRID: AB_2793782

Application(s): ChIP, ChIP-Seq, ELISA, ICC, IF, WB

Reactivity: Human

Quantities: 100 µg, 10 µg

Purification: Ni-NTA

Host: Mouse

Isotype: IgG2a

Concentration: 1 µg/µl

Molecular Weight: 240 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® RNA pol II CTD phospho Ser5 antibody was expressed as full-length IgG with mouse immunoglobulin heavy and light chains (IgG2a isotype) in mammalian 293 cells.

RNA pol II (RNA polymerase II) is responsible for synthesizing messenger RNA in eukaryotes. RNA pol II contains a carboxy terminal domain composed of heptapeptide repeats that are essential for polymerase activity. These repeats contain serine and threonine residues that are phosphorylated in actively transcribing RNA polymerase. In addition, RNA pol II, in combination with several other polymerase subunits, form the DNA binding domain of the polymerase, a groove in which the DNA template is transcribed into RNA. During the transcription cycle, the CTD of the large subunit of RNA pol II is reversibly phosphorylated. RNA pol II containing unphosphorylated CTD is recruited to the promoter, whereas the hyperphosphorylated CTD form is involved in active transcription. Phosphorylation occurs at two sites within the heptapeptide repeat, at serine 2, serine 5 and serine 7. RNA pol II Serine 5 phosphorylation is confined to promoter regions and is necessary for the initiation of transcription.

Immunogen: This RNA pol II CTD phospho Ser5 antibody was raised against a peptide containing the RNA pol II CTD sequence phosphorylated at serine 5.

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

Application Notes:

Applications Validated by Active Motif:

ChIP-Seq: 4 µg each

ICC/IF: 1 - 2 µg/ml

WB*: 0.5 - 2 µg/ml

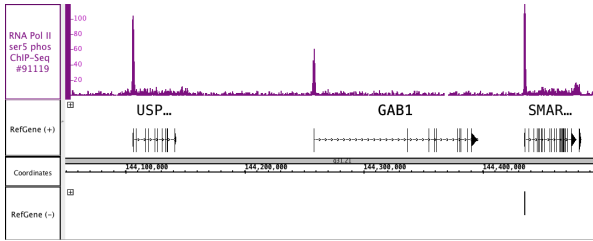
Bead-based ELISA: 0.1 - 5 µg/ml

AbFlex® recombinant antibodies are genetically derived from DNA sequences of parental hybridoma clones. For details on the parental clone, see Catalog No. 61085.

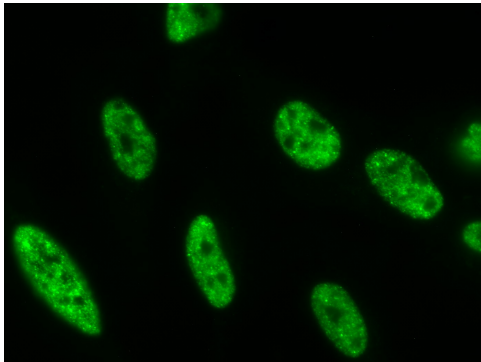
*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western Blot.

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.

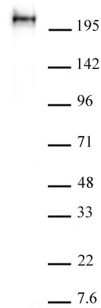
This product is for research use only and is not for use in diagnostic procedures.



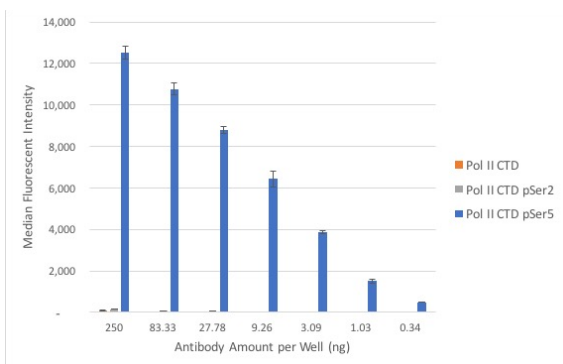
AbFlex[®] RNA pol II CTD phospho Ser5 antibody (rAb) tested by ChIP-Seq. ChIP was performed using the ChIP-IT[®] High Sensitivity Kit (Cat. No. 53040) with 20 µg of chromatin from HL60 cells and 4 µg of AbFlex[®] RNA pol II CTD phospho Ser5 antibody. ChIP DNA was sequenced on the Illumina HiSeq and 20 million sequence tags were mapped to identify RNA pol II phospho Ser5 binding. The image shows binding across a region of chromosome 4.



AbFlex[®] RNA pol II CTD phospho Ser5 antibody (rAb) tested by immunofluorescence. HeLa cell stained with 2µg /mL of AbFlexTM RNA pol II CTD phospho Ser5 antibody (rAb) followed by anti-mouse-IgG-488.



AbFlex[®] RNA pol II CTD phospho Ser5 antibody (rAb) tested by Western blot. HeLa nuclear extract (20 µg per lane) probed with AbFlexTM RNA pol II CTD phospho Ser5 antibody (0.5 µg/ml dilution).



AbFlex[®] RNA pol II CTD phospho Ser5 antibody (rAb) tested by Luminex bead-based specificity analysis.

Luminex bead-based specificity analysis was used to confirm the specificity of AbFlexTM RNA pol II CTD phospho Ser5 antibody (rAb) antibody for RNA pol II CTD peptides. RNA pol II peptides were conjugated to MagPlex Luminex beads and incubated with various amounts of AbFlexTM RNA pol II CTD phospho Ser5 antibody (rAb). Peptide-bound antibody was detected with anti-mouse IgG-Phycoerythrin and read in a Luminex instrument.

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