

RNA pol II CTD phospho Ser5 antibody (pAb)

Catalog Nos: 39749, 39750

RRID: AB_2793330

Isotype: IgG

Application(s): ChIP, ChIP-Seq, WB

Reactivity: Human, Mouse, Wide Range Predicted

Quantities: 100 µg, 10 µg

Purification: Protein A Chromatography

Host: Rabbit

Concentration: 1 µg/µl

Molecular Weight: 210 kDa

Background: 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 5 and Serine 2. 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 synthetic peptide containing the RNA Pol II heptad repeat consensus sequence phosphorylated at serine 5.

Buffer: Purified IgG in PBS (pH 7.5) with 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic. For your convenience, an unpurified serum version (Catalog No. 39233) of this antibody is also available.

Application Notes:

Applications Validated by Active Motif:

ChIP: 4 - 10 µg per ChIP

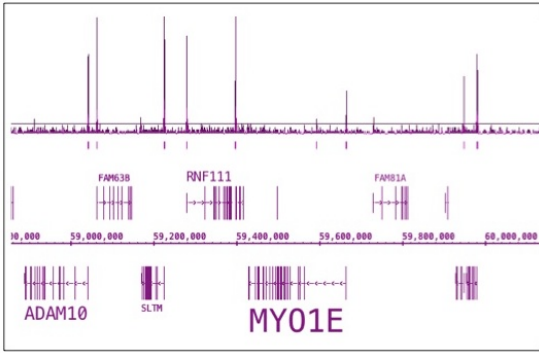
ChIP-Seq: 4 µl each

WB: 0.5 - 2 µg/ml dilution

For RNA pol II CTD phospho Ser5, we also offer AbFlex[®] RNA pol II CTD phospho Ser5 Recombinant Antibody (rAb). For details, see Catalog No. 91119.

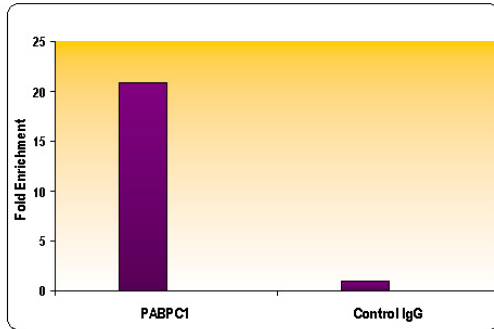
Storage and Guarantee: Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product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

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



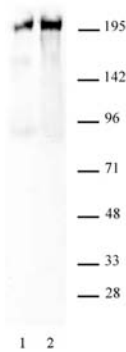
RNA pol II CTD phospho Ser5 antibody (pAb) tested by ChIP-Seq.

ChIP was performed using the ChIP-IT[®] High Sensitivity Kit (Cat. No. 53040) with chromatin from 2.3 million HL-60 cells and 4 ul of antibody. ChIP DNA was sequenced on the Illumina HiSeq and 26 million sequence tags were mapped to identify RNA pol II phospho Ser5 binding. The image shows a 1.2 million base pair region on chromosome 15 with the expected enrichment at gene promoters.



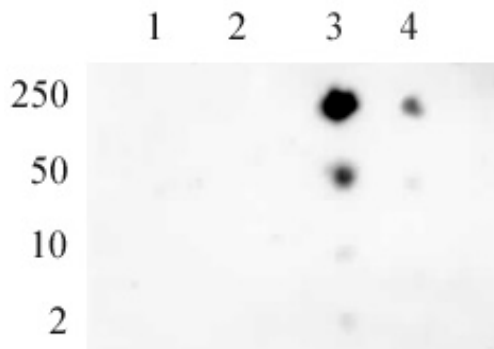
RNA pol II CTD phospho Ser5 antibody tested by ChIP analysis.

Chromatin IP performed using the ChIP-IT[®] Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 10⁶ cell equivalents per ChIP) using 10 µg of RNA pol II CTD phospho Ser5 antibody 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 PABPC1 gene. Data are presented as Fold Enrichment of the ChIP antibody signal versus the negative control IgG (arbitrarily assigned a value of 1) using the ddCT method.



RNA pol II CTD phospho Ser5 antibody tested by Western blot.

Nuclear extract of HeLa cells (20 µg) probed with RNA pol II CTD phospho Ser5 antibody at a dilution of 0.5 µg/ml.



RNA pol II CTD phospho Ser5 pAb tested by dot blot analysis.

Dot blot analysis was used to confirm the specificity of RNA pol II CTD phospho Ser5 antibody for phospho-Ser5 of the RNA Pol II C-terminal domain heptad repeat. Modified and unmodified peptides were spotted onto PVDF and probed with the antibody at a dilution of 0.2 µg/ml. Decreasing amounts of peptide were spotted in each row.

- Lane 1: Peptide phosphorylated at CTD repeat serine 2.
- Lane 2: Unmodified CTD repeat serine 2 peptide.
- Lane 3: Peptide phosphorylated at CTD repeat serine 5.
- Lane 4: Unmodified CTD repeat serine 5 peptide.