

RNA pol II CTD phospho Ser2 antibody (mAb)

Catalog Nos: 61083, 61984, 61084

RRID: AB_2687450

Clone: 3E10

Isotype: IgG1

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

Reactivity: Human, Mouse

Quantities: 100 µg, 50 µg, 10 µg

Purification: Protein G Chromatography

Host: Rat

Concentration: 1 µg/µl

Molecular Weight: 240 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 2, serine 5 and serine 7. RNA pol II phosphorylated at serine 2 is enriched over the gene body and is associated with transcriptional elongation.

Immunogen: This RNA pol II CTD phospho Ser2 antibody was raised against a peptide containing the RNA pol II CTD sequence phosphorylated at serine 2.

Buffer: Purified IgG in 70 mM Tris (pH 8), 105 mM NaCl, 31 mM glycine, 0.07 mM EDTA, 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

Application Notes:

Applications Validated by Active Motif:

WB*: 0.5 - 2 µg/ml

ChIP: 20 µg per ChIP

ChIP-Seq: 20 µg each

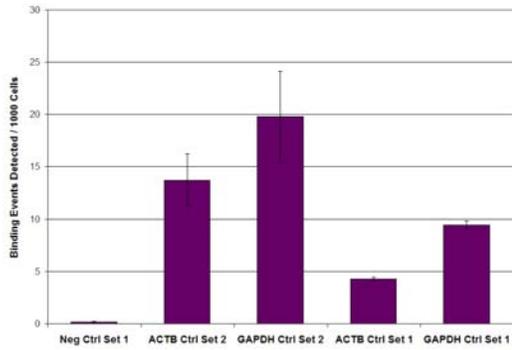
IF: 1:500 dilution

*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.

For RNA pol II CTD phospho Ser2, we also offer AbFlex® RNA pol II CTD phospho Ser2 Recombinant Antibody (rAb). For details, see Catalog No. 91115.

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.

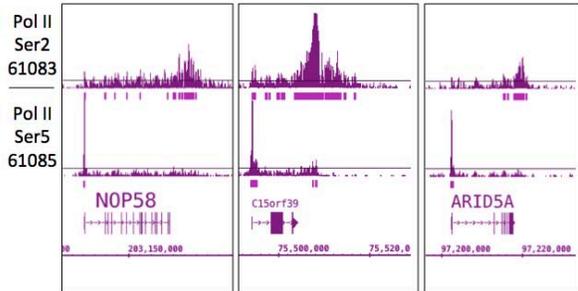


RNA pol II CTD phospho Ser2 antibody (mAb) tested by ChIP.

Chromatin immunoprecipitation (ChIP) was performed using the ChIP-IT[®] High Sensitivity Kit (Cat. No. 53040) with 10 µg of chromatin from human myeloma LP1 cells and 20 µg RNA pol II CTD phospho Ser2 antibody. ChIP DNA was used in qPCR with the control primer pairs or gene-specific primer pairs as indicated. Data are presented as Binding Events Detected per 1000 Cells using Active Motif's Epigenetic Services normalization scheme which accounts for primer efficiency and the amount of chromatin used in the ChIP reaction.

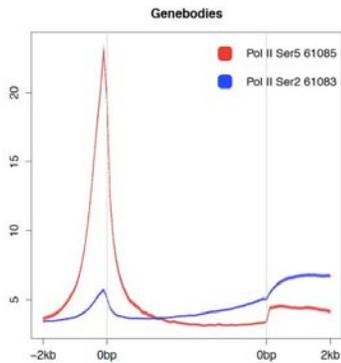
RNA pol II CTD phospho Ser2 antibody (mAb) tested by ChIP-Seq.

ChIP was performed using the ChIP-IT[®] High Sensitivity Kit (Cat. No. 53040) with chromatin from 2.3 million HL-60 cells and 10 µg of antibody. ChIP DNA was sequenced on the Illumina HiSeq and 22 million sequence tags were mapped to identify RNA pol II phospho Ser2 binding. Data is compared to ChIP-Seq data using a phospho Ser5 antibody (61085). ChIP-Seq data from three specific genes is shown as an example. The Pol II phospho Ser2 antibody detects polymerase more toward the 3' end of the genes and the phospho Ser5 antibody detects Pol II more at the 5' end of the genes.



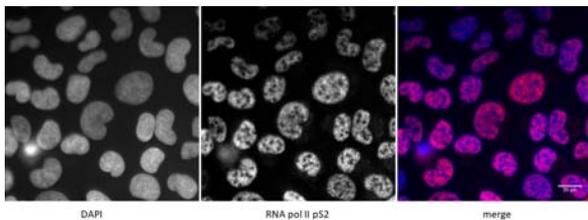
RNA pol II CTD phospho Ser2 antibody (mAb) tested by ChIP-Seq.

ChIP-Seq was performed in a bladder cancer cell line using RNA pol II CTD phospho Ser5 antibody (Cat. No. 61085) and RNA pol II CTD phospho Ser2 antibody (Cat. No. 61083). The average ChIP-Seq signal across all genes is shown in the graphic. As expected Pol II phosphoserine 5 is enriched at promoters and phosphoserine 2 is enriched toward the 3' end of genes.



Detection of RNA Pol II pS2 by immunofluorescence.

U2OS cells were stained with RNA Pol II pS2 antibody at a dilution of 1:500. Left panel: DAPI. Middle panel: RNA Pol II pS2 antibody staining. Right panel: merge.



RNA pol II phospho Ser2 antibody (mAb) tested by Western blot.

HeLa nuclear extract (20 µg per lane) probed with RNA pol II phospho Ser2 antibody (mAb) at a 1 µg/ml dilution.

