RNA pol II CTD phospho Ser5 antibody (mAb)

Catalog Nos: 61085, 61986, 61086
RRID: AB_2687451
Clone: 3E8
Isotype: IgG2a
Application(s): ChIP, ChIP-Seq, ICC, IF, IP, WB
Reactivity: Human

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 Serine 5 phosphorylation** is confined to promoter regions and is necessary for the initiation of transcription.

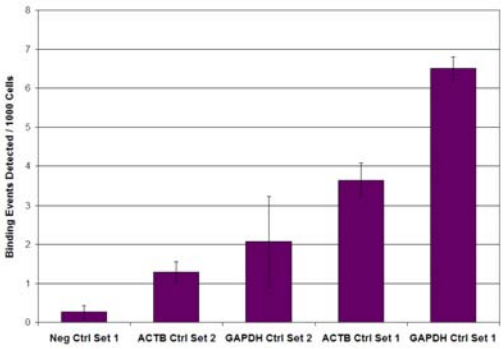
Immunogen: This RNA pol II CTD phospho Ser5 antibody was raised against a peptide containing the RNA pol II CTD sequence phosphorylated at serine 5.

Buffer: Purified IgG in 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.

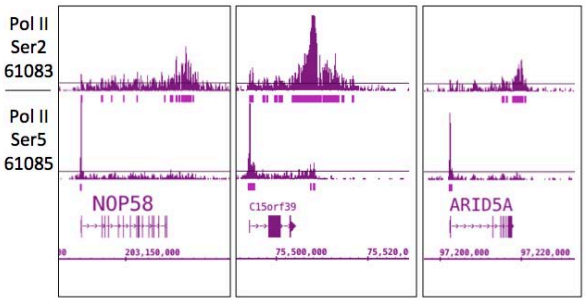
Application Notes:
Applications Validated by Active Motif:
ChIP: 5-10 µg per ChIP
ChIP-Seq: 5-10 µg each
IF: 1:500 dilution
WB*: 0.5 - 2 µg/ml dilution

*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. In addition, we recommend the addition of 0.05% Tween 20 to all blocking solutions to reduce background.

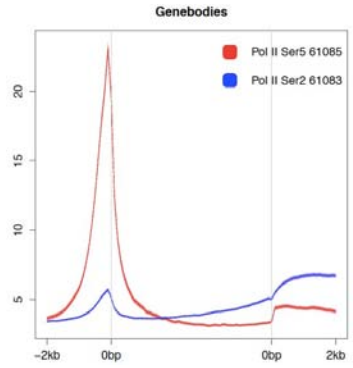
Storage and Guarantee: Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product 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 (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 10 µg RNA pol II CTD phospho Ser5 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.

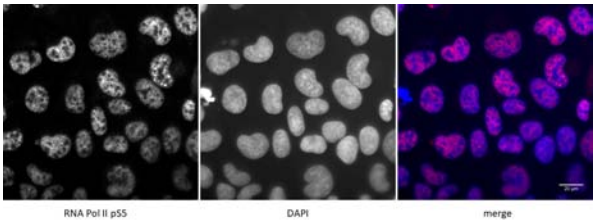


RNA pol II CTD phospho Ser5 antibody (mAb) tested by ChIP-Seq.
ChIP was performed using the ChIP-IT® High Sensitivity Kit (Cat. No. 53040) with chromatin from 2.3 million HL60 cells and 5 ug of antibody. ChIP DNA was sequenced on the Illumina HiSeq and 23 million sequence tags were mapped to identify RNA pol II phospho Ser5 binding. Data is compared to ChIP-Seq data using a phospho Ser2 antibody (61083). ChIP-Seq data from three specific genes is shown as an example. The Pol II phospho Ser5 antibody detects polymerase more at the 5´ end of the genes and the phospho Ser2 antibody detects Pol II more toward the 3´ end of the genes.

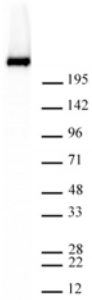


RNA pol II CTD phospho Ser5 antibody (mAb) tested by ChIP-Seq.
ChIP-Seq was performed in a bladder cancer cell line using RNA pol II CTD phospho Ser5 antibody (Cat. No. 61085) and RNA pol II CTD phospho Ser2 antibody (Cat. No. 61083). The average ChIP-Seq signal across all genes is shown in the graphic. 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 pS5 by immunofluorescence



U2OS cells were stained with RNA Pol II pS5 antibody at a dilution of 1:500. Left panel: RNA Pol II pS5 antibody staining. Middle panel:DAPI. Right panel: merge.



RNA pol II CTD phospho Ser5 mAb tested by Western blot.
20 µg of HeLa nuclear extract was run on SDS-PAGE and probed with antibody at 2 µg/ml.