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Recombinant Anti-Rpb1 CTD (Phospho-S5) Rabbit mAb

Catalog # AP95043

Product Information

Application	WB, IHC, FC, IP, IF/IC
Primary Accession	P24928
Reactivity	Human, Mouse, Rat
Host	Rabbit
Clonality	Monoclonal
Calculated MW	217206

Additional Information

Gene ID	5430
Other Names	POLR2; DNA-directed RNA polymerase II subunit RPB1; RNA polymerase II subunit B1; DNA-directed RNA polymerase II subunit A; DNA-directed RNA polymerase III largest subunit; RNA-directed RNA polymerase II subunit RPB1
Target/Specificity	KLH-conjugated synthetic Phospho-peptide corresponding to residues surrounding S5 of human Rpb1 CTD. The exact sequence is proprietary.
Dilution	WB~~WB (1/500 - 1/1000) IHC~~1:100~500 FC~~1:10~50 IP~~N/A IF/IC~~N/A
Format	Liquid in PBS, pH 7.3, 50% glycerol, 0.05% BSA, and 0.05% Proclin300.
Storage	Store at -20 °C.Stable for 12 months from date of receipt

Protein Information

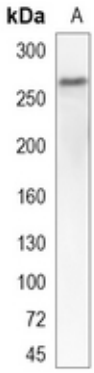
Name	POLR2A (HGNC:9187)
Synonyms	POLR2
Function	Catalytic core component of RNA polymerase II (Pol II), a DNA-dependent RNA polymerase which synthesizes mRNA precursors and many functional non-coding RNAs using the four ribonucleoside triphosphates as substrates (By similarity) (PubMed:23748380, PubMed:27193682, PubMed:30190596, PubMed:9852112). Pol II-mediated transcription cycle proceeds through transcription initiation, transcription elongation and transcription termination stages. During transcription initiation, Pol II pre-initiation complex (PIC) is recruited to DNA promoters, with focused-type promoters containing either the initiator (Inr) element, or the TATA-box found in cell-type specific genes and dispersed-type promoters that often contain hypomethylated CpG islands usually found in housekeeping genes. Once the polymerase has escaped from the promoter it enters the elongation phase during which RNA is actively polymerized, based on complementarity with the template DNA strand.

Transcription termination involves the release of the RNA transcript and polymerase from the DNA (By similarity) (PubMed:23748380, PubMed:27193682, PubMed:28108474, PubMed:30190596, PubMed:9852112). Forms Pol II active center together with the second largest subunit POLR2B/RPB2. Appends one nucleotide at a time to the 3' end of the nascent RNA, with POLR2A/RPB1 most likely contributing a Mg(2+)-coordinating DxDGD motif, and POLR2B/RPB2 participating in the coordination of a second Mg(2+) ion and providing lysine residues believed to facilitate Watson-Crick base pairing between the incoming nucleotide and template base. Typically, Mg(2+) ions direct a 5' nucleoside triphosphate to form a phosphodiester bond with the 3' hydroxyl of the preceding nucleotide of the nascent RNA, with the elimination of pyrophosphate. The reversible pyrophosphorolysis can occur at high pyrophosphate concentrations (By similarity) (PubMed:30190596, PubMed:8381534, PubMed:9852112). Can proofread the nascent RNA transcript by means of a 3' -> 5' exonuclease activity. If a ribonucleotide is mis-incorporated, backtracks along the template DNA and cleaves the phosphodiester bond releasing the mis-incorporated 5'-ribonucleotide (By similarity) (PubMed:8381534). Through its unique C-terminal domain (CTD, 52 heptapeptide tandem repeats) serves as a platform for assembly of factors that regulate transcription initiation, elongation and termination. CTD phosphorylation on Ser-5 mediates Pol II promoter escape, whereas phosphorylation on Ser-2 is required for Pol II pause release during transcription elongation and further pre-mRNA processing. Additionally, the regulation of gene expression levels depends on the balance between methylation and acetylation levels of the CTD-lysines. Initiation or early elongation steps of transcription of growth-factor-induced immediate early genes are regulated by the acetylation status of the CTD. Methylation and dimethylation have a repressive effect on target genes expression. Cooperates with mRNA splicing machinery in co-transcriptional 5'-end capping and co-transcriptional splicing of pre-mRNA (By similarity) (PubMed:24207025, PubMed:26124092).

Cellular Location

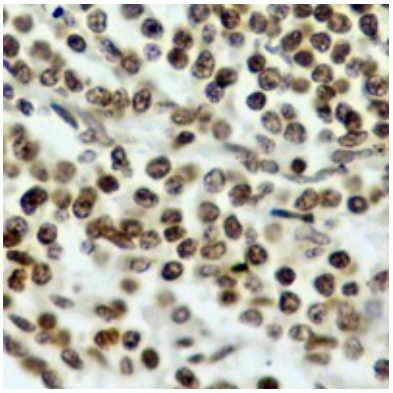
Nucleus. Cytoplasm. Chromosome. Note=Hypophosphorylated form is mainly found in the cytoplasm, while the hyperphosphorylated and active form is nuclear (PubMed:26566685). Co-localizes with kinase SRPK2 and helicase DDX23 at chromatin loci where unscheduled R-loops form (PubMed:28076779).

Images

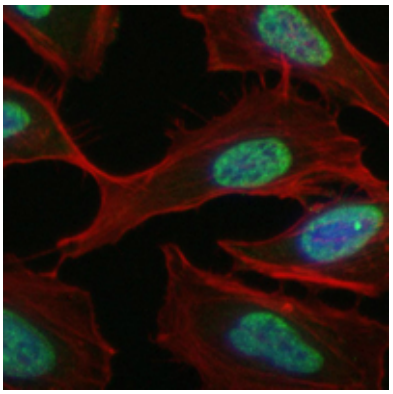


Western blot analysis of Rpb1 CTD (Phospho-S5) expression in HeLa (A) whole cell lysates. (Predicted band size: 217 kD; Observed band size: 270 kD)

Immunohistochemical analysis of Rpb1 CTD (Phospho-S5) staining in human spleen formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0). The section was then incubated with the



antibody at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Immunofluorescent analysis of Rpb1 CTD (Phospho-S5) staining in HeLa cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with the primary antibody in 3% BSA-PBS and incubated overnight at 4 °C in a hidified chamber. Cells were washed with PBST and incubated with an AF488-conjugated secondary antibody (green) in PBS at room temperature in the dark. Phalloidin - AF594 was used to stain Actin filaments (red). DAPI was used to stain the cell nuclei (blue).

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