abcam

ab5401

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [8A7]

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	Preservative: 0.02% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Synthetic Peptide within Human POLR2A pS5. The exact immunogen used to generate this antibody is proprietary information. Database link P24928
Clone number	8A7
Purification technique	Affinity purification Protein A
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Predicted

Species	Mouse
Dilution info	-
Notes	-

Target data

See full target information POLR2A pS5

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). RNA-dependent RNA polymerase that catalyzes the extension of a non-coding RNA (ncRNA) at the 3'-end using the four ribonucleoside triphosphates as substrates. An internal ncRNA sequence near the 3'-end serves as a template in a single-round Pol II-mediated RNA polymerization reaction. May decrease the stability of ncRNAs that repress Pol II-mediated gene transcription. (Microbial infection) Acts as an RNA-dependent RNA polymerase when associated with small delta antigen of Hepatitis delta virus, acting both as a replicase and transcriptase for the viral RNA circular genome.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	RNA polymerase II CTD repeat YSPTSPS also known as the C-terminal domain of RNA polymerase II is a critical component of the RNA polymerase II enzyme commonly referred to as pol II. This domain is characterized by the repetitive sequence YSPTSPS which plays a significant role in the regulation of transcription. The mass of RNA polymerase II including its CTD varies but is essential for its function in gene expression. RNA polymerase II with the CTD is expressed in the nucleus of eukaryotic cells where it orchestrates the transcription of DNA into mRNA.
Biological function summary	RNA polymerase II CTD repeat YSPTSPS is essential for the transcription progression from initiation to termination. It is part of the large RNA polymerase II complex interacting with various transcription factors and enzymes necessary for RNA processing. The phosphorylation state of the CTD particularly on serine residues regulates interactions with splicing machinery and other RNA processing factors. This modulation ensures the coupling between transcription and RNA processing events controlling mRNA synthesis and maturation.
Pathways	RNA polymerase II CTD repeat YSPTSPS is important in the mRNA synthesis pathway specifically in transcriptional regulation and processing of nascent RNA transcripts. It interacts with proteins such as the transcription factors TFIIH and TFIIB which aid in promoter recognition and open complex formation. The CTD's dynamic phosphorylation pattern allows integration into multiple cellular

pathways most importantly connecting transcription with RNA splicing and transport pathways.

Associated diseases and disorders

Abnormal function or mutations in RNA polymerase II CTD repeat YSPTSPS associate with diseases such as transcription-related syndromes and certain cancers. Deficient CTD phosphorylation can lead to improper mRNA processing resulting in neural developmental disorders. Additionally its interaction with proteins like CDK7 which phosphorylates the CTD links it to tumors where transcriptional dysregulation is a hallmark. Understanding the CTD's role in these diseases provides insight into therapeutic targets and strategies for intervention.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

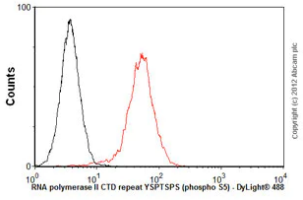
We do not recommend this combination. It is not covered by our product promise.

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2 product images



Flow Cytometry - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [8A7] (ab5401)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Flow Cytometry staining using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with ab5401 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10%

normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab5401, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

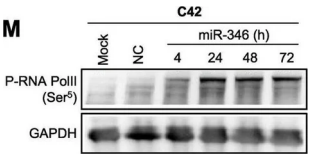


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Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [8A7] (ab5401)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Western blot staining using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

Western Blotting using Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [8A7], ab5401. Publication image from Liu, Y. et al., 2022, Mol Cancer, 35317841. Legend direct from paper.

miR-346 Induces Genome-Wide Double Strand DNA Breaks at Transcription Start Sites. A Schematic illustration of INDUCE-seq protocol, B Quantification of number of ds DNA breaks identified per ng of sequencing library, C Genome browser comparison of number of breaks per kb across chromosome 11 for C42, C42/NC, C42/miR-346 and C42/shNORAD cells treated ± Dox. Peak height corresponds to number of DNA breaks. Similar results were obtained for other chromosomes. D, E Integration of miR-346 and shNORAD dsDNA break coordinates with D) annotated transcription start sites (TSSs - hg19 refseq gene list) and E) PC ChIP-seq-identified CTCF, ZFX and histone H3K27ac sites. F) top 1000 genes expressed in C42 cells show greater enrichment of miR-346-induced dsDNA breaks at their TSSs (dark red) compared to total C42-expressed genes (yellow), G enrichment of miR-346 dsDNA breaks at TSSs of miR-346-downregulated (dark blue), but not –upregulated (dark green), genes. H Enrichment of miR-346 DSBs at (i) ChIP-seq-identified AR binding sites (LNCaP cells - pink) and (ii) canonical AREs (consensus ARE sequence from JASPAR [http://jaspar.genereg.net/]- blue). miR-346 DSB enrichment at TSSs from hg19 refseq gene list is shown for reference. J, K, L Integration of miR-346 dsDNA break coordinates with PC ChIP-seq-identified binding sites for (J) PC-implicated TFs/ pioneer factors cMyc (GSM1907204), CTCF (GSM2827203), ETV1 (GSM1145322), GATA2 (GSM941195), FOXA1 (GSM1863005), HOXB13 (GSM2480818), NKX3.1 (GSM989640) and POL2RA (GSM696843) (K) AR-related Nuclear Receptor family members PR (GSM50151), ESR1 (GSE43988) and NR3C1 (GR) (GSM759669) and (L) neuroendocrine PC-associated TFs nMyc (GSM2305251), EZH2 (GSM2305255) and RUNX2 (GSM838400). M Western blot analysis of phospho-Ser5-RNA PolII protein levels in C42 cells transfected with miR-346 or NC (20 nM) for indicated durations. GAPDH was used as a control for loading and a representative image is shown. See also Fig. S11

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