abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody ab5131

48 Abreviews 421 References 7 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody
Description	Rabbit polyclonal to RNA polymerase II CTD repeat YSPTSPS (phospho S5)
Host species	Rabbit
Specificity	This antibody recognises the phosphorylated serine found in the amino acid 5 position of the C-terminal domain repeat YSPTSPS of RNA polymerase II. From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help.
Tested applications	**Suitable for:** IHC-Fr, IHC-P, ELISA, IP, WB, ChIP, ICC/IF, IHC - Wholemount
Species reactivity	**Reacts with:** Mouse, Rat, Human, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Zebrafish **Predicted to work with:** a wide range of other species
Immunogen	**This product was produced with the following immunogens:** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab18488**) Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab18488**) Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab18488**) Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab18488**)
Positive control	ICC/IF: HeLa cells. WB: Xenopus laevis whole tissue lysate
General notes	Phosphorylation of RNA polymerase II's largest subunit C-terminal domain (CTD) is a key event during mRNA metabolism. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies

and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Primary antibody notes	Phosphorylation of RNA polymerase II's largest subunit C-terminal domain (CTD) is a key event during mRNA metabolism.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5131 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-Fr		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
ELISA	★★★★☆ (1)	Use at an assay dependent concentration.
IP	★★★★☆ (1)	Use at an assay dependent concentration.
WB	★★★★★ (15)	1/1000. Detects a band of approximately 240 kDa (predicted molecular weight: 217 kDa).Can be blocked with **Human RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide (ab18488)**.
ChIP	★★★★☆ (17)	Use 2 µg for 25 µg of chromatin.
ICC/IF	★★★★☆ (9)	Use a concentration of 1 µg/ml.

Application	Abreviews	Notes
IHC - Wholemount	★★★★★ (2)	Use at an assay dependent concentration.

Target

Function

DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Largest and catalytic component of RNA polymerase II which synthesizes mRNA precursors and many functional non-coding RNAs. Forms the polymerase active center together with the second largest subunit. Pol II is the central component of the basal RNA polymerase II transcription machinery. It is composed of mobile elements that move relative to each other. RPB1 is part of the core element with the central large cleft, the clamp element that moves to open and close the cleft and the jaws that are thought to grab the incoming DNA template. At the start of transcription, a single-stranded DNA template strand of the promoter is positioned within the central active site cleft of Pol II. A bridging helix emanates from RPB1 and crosses the cleft near the catalytic site and is thought to promote translocation of Pol II by acting as a ratchet that moves the RNA-DNA hybrid through the active site by switching from straight to bent conformations at each step of nucleotide addition. During transcription elongation, Pol II moves on the template as the transcript elongates. Elongation is influenced by the phosphorylation status of the C-terminal domain (CTD) of Pol II largest subunit (RPB1), which serves as a platform for assembly of factors that regulate transcription initiation, elongation, termination and mRNA processing. Acts as an RNA-dependent RNA polymerase when associated with small delta antigen of Hepatitis delta virus, acting both as a replicate and transcriptase for the viral RNA circular genome.

Sequence similarities

Belongs to the RNA polymerase beta' chain family.

Domain

The C-terminal domain (CTD) serves as a platform for assembly of factors that regulate transcription initiation, elongation, termination and mRNA processing.

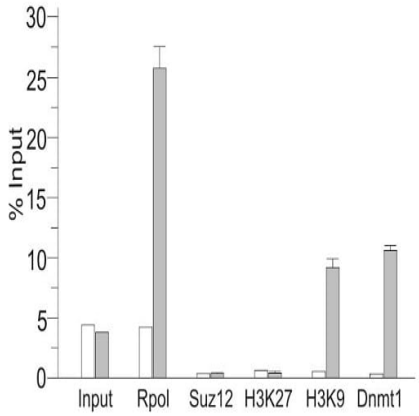
Post-translational modifications

The tandem heptapeptide repeats in the C-terminal domain (CTD) can be highly phosphorylated. The phosphorylation activates Pol II. Phosphorylation occurs mainly at residues 'Ser-2' and 'Ser-5' of the heptapeptide repeat and is mediated, at least, by CDK7 and CDK9. CDK7 phosphorylation of POLR2A associated with DNA promotes transcription initiation by triggering dissociation from DNA. Phosphorylation also takes place at 'Ser-7' of the heptapeptide repeat, which is required for efficient transcription of snRNA genes and processing of the transcripts. The phosphorylation state is believed to result from the balanced action of site-specific CTD kinases and phosphatases, and a 'CTD code' that specifies the position of Pol II within the transcription cycle has been proposed. Dephosphorylated by the protein phosphatase CTDSP1.
Among tandem heptapeptide repeats of the C-terminal domain (CTD) some do not match the Y-S-P-T-S-P-S consensus, the seventh serine residue 'Ser-7' being replaced by a lysine. 'Lys-7' in these non-consensus heptapeptide repeats can be alternatively acetylated, methylated and dimethylated. EP300 is one of the enzyme able to acetylate 'Lys-7'. Acetylation at 'Lys-7' of non-consensus heptapeptide repeats is associated with 'Ser-2' phosphorylation and active transcription. It may regulate initiation or early elongation steps of transcription specially for inducible genes.
Methylated at Arg-1810 prior to transcription initiation when the CTD is hypophosphorylated, phosphorylation at Ser-1805 and Ser-1808 preventing this methylation. Symmetrically or asymmetrically dimethylated at Arg-1810 by PRMT5 and CARM1 respectively. Symmetric or asymmetric dimethylation modulates interactions with CTD-binding proteins like SMN1/SMN2 and TDRD3. SMN1/SMN2 interacts preferentially with the symmetrically dimethylated form while

TDRD3 interacts with the asymmetric form. Through the recruitment of SMN1/SMN2, symmetric dimethylation is required for resolving RNA-DNA hybrids created by RNA polymerase II, that form R-loop in transcription terminal regions, an important step in proper transcription termination. CTD dimethylation may also facilitate the expression of select RNAs. Among tandem heptapeptide repeats of the C-terminal domain (CTD) some do not match the Y-S-P-T-S-P-S consensus, the seventh serine residue 'Ser-7' being replaced by a lysine. 'Lys-7' in these non-consensus heptapeptide repeats can be alternatively acetylated, methylated and dimethylated. Methylation occurs in the earliest transcription stages and precedes or is concomitant to 'Ser-5' and 'Ser-7' phosphorylation.
Ubiquitinated by WWP2 leading to proteasomal degradation (By similarity). Following UV treatment, the elongating form of RNA polymerase II (RNA pol IIo) is ubiquitinated UV damage sites without leading to degradation: ubiquitination is facilitated by KIAA1530/UVSSA and promotes RNA pol IIo backtracking to allow access to the nucleotide excision repair machinery.

Cellular localization Nucleus.

Images

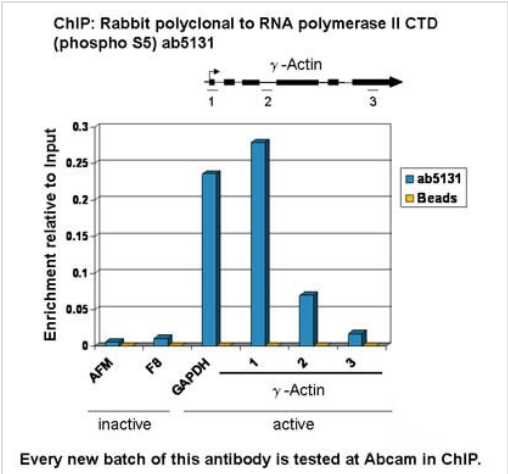


ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (ab5131)

Murgatroyd et al PLoS One. 2014 Mar 5;9(3):e90277. doi: 10.1371/journal.pone.0090277. eCollection 2014. Fig S2. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Chromatin from cultured cells, mouse PVN punches (individual pools formed from groups of 3) or whole hypothalami dissected from fresh brains were cross linked, disrupted by sonification and purified.

The binding of Suz12 to wingless (*Wnt1*), a beta-catenin dependent developmental regulator and to the RNA polymerase II promoter (*RNAPII*), a housekeeping gene, served as positive and negative controls, respectively, in these experiments.

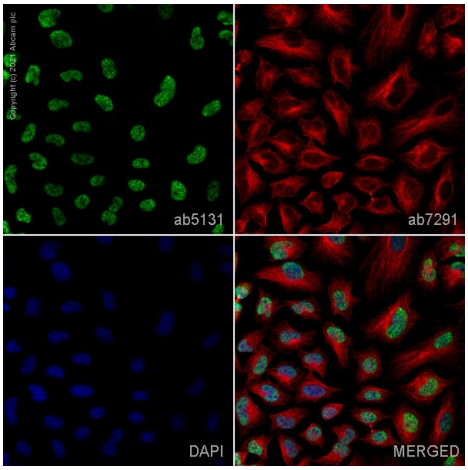


ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (ab5131)

Chromatin was prepared from U-2 OS (Human bone osteosarcoma epithelial cell line) cells according to the Abcam X-ChIP protocol.

Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 2 µg of ab5131 (blue), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified on the inactive AFM and F8 promoters, the GAPDH promoter (active) and over the y-Actin gene (active).

Schematic diagram of the y-Actin gene is shown on the top of the figure. Black boxes represent exons and thin lines represent introns. PCR products are depicted as bars under the gene.

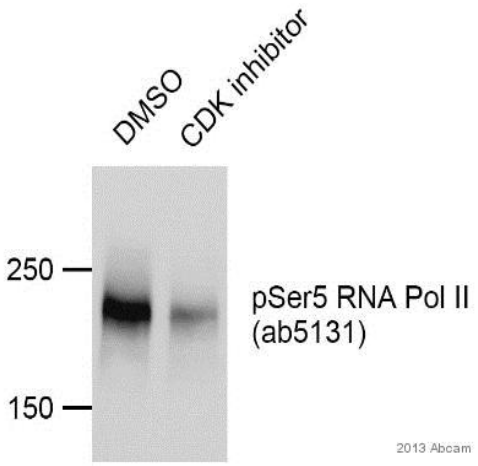


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody (ab5131)

ab5131 staining RNA polymerase II CTD repeat YSPTSPS (phospho S5) in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab5131 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (ab5131)

This image is courtesy of an anonymous Abreview

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody (ab5131) at 1/5000 dilution

Lane 1 : Xenopus laevis whole tissue lysate treated with DMSO for 24 hours
Lane 2 : Xenopus laevis whole tissue lysate treated with CDK inhibitor for 24 hours

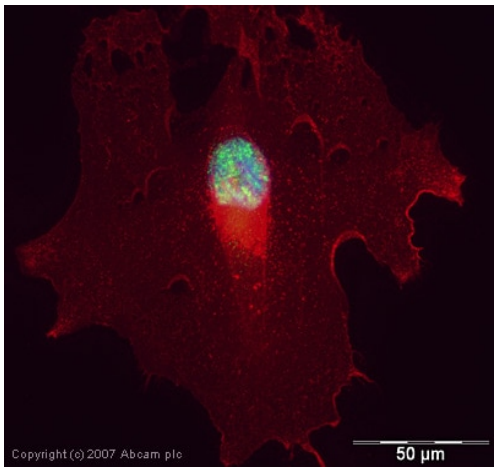
Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG polyclonal at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 217 kDa
Observed band size: 240 kDa

Exposure time: 1 minute

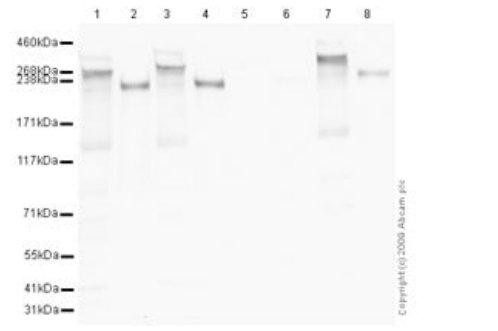


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (ab5131)

ICC/IF image of ab5131 stained HeLa(Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed in methanol for 5 minutes, permabilized in TBS-T for 20 minutes and incubated with the antibody (ab5131, 1 µg/ml) for 1 hour at room temperature. 1% BSA / 10% normal goat serum / 0.3M glycine was used to quench auto-fluorescence and block non-specific protein-protein interactions.

The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1 hour. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (ab5131)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody (ab5131) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate
Lane 2 : S.cerevisiae (Y190) Whole Cell Lysate
Lane 3 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS peptide (**ab12795**) at 1 µg/ml
Lane 4 : S.cerevisiae (Y190) Whole Cell Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS peptide (**ab12795**) at 1 µg/ml
Lane 5 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate with Human RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide (**ab18488**) at 1 µg/ml
Lane 6 : S.cerevisiae (Y190) Whole Cell Lysate with Human RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide (**ab18488**) at 1 µg/ml
Lane 7 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide (**ab12793**) at 1 µg/ml
Lane 8 : S.cerevisiae (Y190) Whole Cell Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide (**ab12793**) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

Secondary

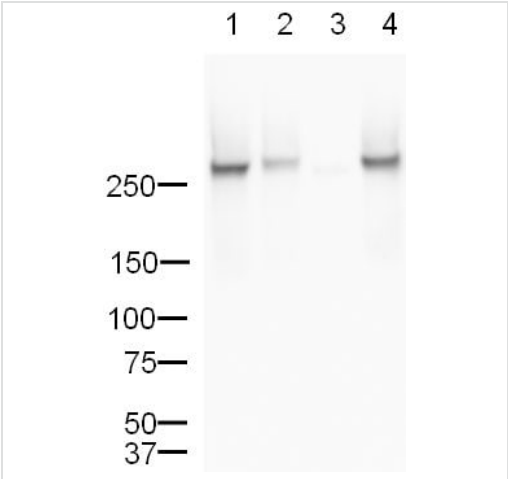
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 217 kDa

Exposure time: 30 seconds



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (ab5131)

Lanes 1 & 3-4 : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody (ab5131) at 1/500 dilution
Lane 2 : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody (ab5131) at 1/2000 dilution

Lanes 1-2 : HeLa nuclear extract
Lane 3 : HeLa nuclear extract with Human RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide (**ab18488**) at 1 µg/ml
Lane 4 : HeLa nuclear extract with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS peptide (**ab12795**) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 217 kDa
Observed band size: 250 kDa

Exposure time: 30 seconds

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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