# Product datasheet

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

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<td><strong>Product name</strong></td>
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<td><strong>Description</strong></td>
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<td><strong>Host species</strong></td>
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<td><strong>Specificity</strong></td>
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<td><strong>Predicted to work with:</strong> a wide range of other species</td>
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### Immunogen

Synthetic peptide of *Saccharomyces cerevisiae* RNA polymerase II CTD repeat YSPTSPS, phosphorylated at S5. Read Abcam's proprietary immunogen policy (Peptide available as [ab18488](https://www.abcam.com/)).

### Positive control

This antibody gave a positive signal in *S.cerevisiae* (Y190) Whole Cell Lysate and HeLa Nuclear Lysate within Western blot, HeLa whole cell lysate within Immunofluorescence and Human urinary bladder tissue within Immunohistochemistry.

### General notes

Phosphorylation of RNA polymerase II's largest subunit C-terminal domain (CTD) is a key event during mRNA metabolism.

## 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

Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4

### 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

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.

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<th>Abreviews</th>
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<tr>
<td>IP</td>
<td></td>
<td>1/50.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>ELISA</td>
<td></td>
<td>1/438.</td>
</tr>
<tr>
<td>IHC - Wholemount</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>CHIPseq</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>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).</td>
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<tr>
<td>ChiP</td>
<td></td>
<td>Use 2-25 µg for µg of chromatin.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 1 µg/ml.</td>
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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**
Chromatin was prepared from U2OS cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 mins. 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 γ-Actin gene (active). Schematic diagram of the γ-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.

Lanes 1 & 3-4: Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (ab5131) at 1/500 dilution
Lane 2: Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (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
Western blot using ab5131.

Lane 1: ab5131 at 1/500
Lane 2: ab5131 at 1/2000
Lane 3: ab5131 at 1/500 blocked with phospho peptide
Lane 4: ab5131 at 1/500 blocked with non-phospho control peptide

Phospho peptide is YSPTSpSYSPTSpPS-GGC (ab18488)
Non-phospho control peptide is YSPTSPSYSPTPS-GGC (ab12795)

Secondary ab: Goat Anti-Rabbit IgG H&L (HRP) (ab6721) secondary antibody (1/5000)
Lanes 1 to 4: 20µg of HeLa nuclear extract per lane
Blocking peptides used at 1µg/ml.
Exposure time: 30 seconds
Expected molecular weight: ~240 kDa

IHC - Wholemount of Caenorhabditis elegans larvae labelling RNA polymerase II CTD repeat YSPTPS (phospho S5) with ab5131.
The sample was incubated with primary antibody (1/500 in PBS + 3% BSA + 0.1% Triton X-100) for 12 hours at 4°C. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody (1/1000), was used as the secondary antibody.
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 - ChIP Grade (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
HeLa cells were fixed with 4% formaldehyde in PEM buffer. The coverslip was incubated in blocking buffer of 5% powdered milk in TBS-T plus 0.02% sodium azide for 1 hour at room temperature. Blocking buffer was removed and primary antibody was added at a dilution of 1/160 and incubated overnight at 4 degrees celsius. The coverslips were then washed 4-5 times with blocking buffer for 5 minutes.

Secondary antibody, goat anti-rabbit Alexa 594, was added at a dilution of 1/1000 and incubated at room temperature for one hour. From this point on coverslips were covered with foil to protect them from light. They were washed 5 times with TBS-T and then one time with PEM, for 5 minutes each wash. The coverslips were fixed 10-30 minutes in 4% formaldehyde in PEM buffer, then washed 3 times with PEM buffer for 5 minutes. 0.1M ammonium chloride in PEM buffer was added for 10 minutes to quench auto-fluorescence, and then slips were washed 2 times for 5 minutes in PEM followed by 3 washes for 5 minute.

ICC/IF image of ab5131 stained human HeLa cells. The cells were methanol fixed (5 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab5131, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to quench autofluorescence 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 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).

All lanes: Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (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 S1606 + S1613) peptide (ab12793) at 1 µg/ml

**Lane 8**: S.cerevisiae (Y190) Whole Cell Lysate with *S. cerevisiae* RNA polymerase II CTD repeat YSPTSPS (phospho S1606 + S1613) 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
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody - ChIP Grade (ab5131)

(Image courtesy of Human Protein Atlas)

ab5131 staining RNA Polymerase in Human urinary bladder tissue. Paraffin embedded human skin tissue was incubated with ab5131 for 30 mins at room temperature. Antigen retrieval was performed by heat induction in citrate buffer pH 6. ab5131 was tested in a tissue microarray (TMA) containing a wide range of normal and cancer tissues as well as a cell microarray consisting of a range of commonly used, well characterised human cell lines. Further results for this antibody can be found at www.proteinatlas.org

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