

## Product datasheet

# Anti-RNA polymerase II CTD repeat YSPTSPS antibody [8WG16] - ChIP Grade ab817

★★★★☆ 38 Abreviews 197 References 6 Images

### Overview

<b>Product name</b>	Anti-RNA polymerase II CTD repeat YSPTSPS antibody [8WG16] - ChIP Grade
<b>Description</b>	Mouse monoclonal [8WG16] to RNA polymerase II CTD repeat YSPTSPS - ChIP Grade
<b>Host species</b>	Mouse
<b>Tested applications</b>	<b>Suitable for:</b> CHIPseq, WB, ICC/IF, IHC - Wholemount, ChIP, Flow Cyt, IP
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Cow, Human, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Quail, Neurospora crassa, Rice <b>Predicted to work with:</b> a wide range of other species
<b>Immunogen</b>	Other Immunogen Type corresponding to RNA polymerase II CTD repeat YSPTSPS. Immunogen: Wheat germ RNA Polymerase II
<b>General notes</b>	This antibody may be used to detect unproteolyzed RNA polymerase II or to inhibit specific transcription from class II promoters. This is a polyol-responsive antibody and can be used in gentle purifications of RNA polymerase II from from wheat germ, calf thymus, and yeast and is likely to purify RNA polymerase II from most eukaryotic organisms. It inhibits promoter-directed transcription, but it does not inhibit elongation in the nonspecific transcription assay.

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	Constituent: PBS
<b>Purity</b>	Affinity purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	8WG16
<b>Isotype</b>	IgG2a

### Applications

Our [Abpromise guarantee](#) covers the use of **ab817** 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
CHIPseq		Use at an assay dependent concentration. PubMed: 19251593 Use 2ug per 0.3ml of sonicated chromatin.
WB	★★★★★	Use a concentration of 0.25 - 2 µg/ml. Detects a band of approximately 217 kDa (predicted molecular weight: 217 kDa).
ICC/IF	★★★★★	Use a concentration of 1 - 5 µg/ml.
IHC - Wholemount	★★★★★	Use at an assay dependent concentration. See Abreview.
ChIP	★★★★★	Use at an assay dependent concentration. See Abreview; the user recommends using anti-mouse IgG coated Dynabeads instead of Protein A to recover the precipitate).
Flow Cyt		Use 0.5µg for 10 <sup>6</sup> cells. <a href="#">ab170191</a> - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody. We recommend using <a href="#">Goat Anti-Mouse IgG H&amp;L (DyLight® 488) preadsorbed (ab96879)</a> secondary antibody.
IP	★★★★★	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.

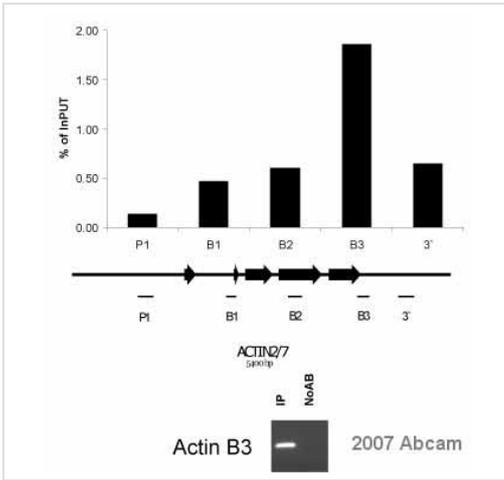
Methylated at Arg-1810 prior to transcription initiation when the CTD is hypophosphorylated, phosphorylation at Ser-1805 and Ser-1808 preventing this methylation. Symmetrically or asymmetrically dimethylated at Arg-1810 by PRMT5 and CARM1 respectively. Symmetric or asymmetric dimethylation modulates 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 Ilo) is ubiquitinated UV damage sites without leading to degradation: ubiquitination is facilitated by KIAA1530/UVSSA and promotes RNA pol Ilo backtracking to allow access to the nucleotide excision repair machinery.

## Cellular localization

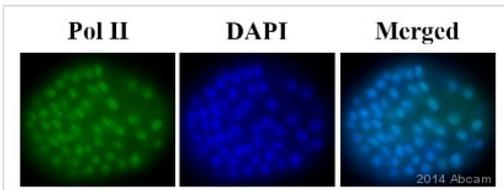
Nucleus.

## Images



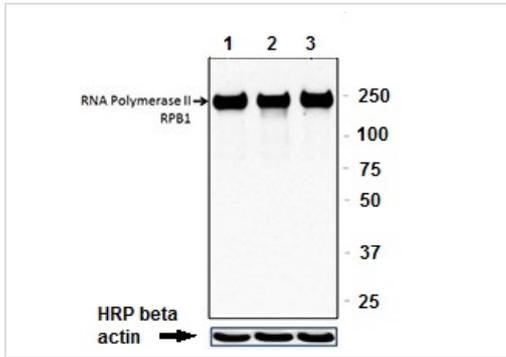
ChIP - Anti-RNA polymerase II CTD repeat  
YSPTSPS antibody [8WG16] - ChIP Grade (ab817)  
This image is courtesy of an Abreview submitted by Dr  
Szymon Świeżewski

Various regions across the Actin2/7 loci were tested for the presence of RNA polymerase II CTD repeat YSPTSPS. A nuclear lysate from *Arabidopsis thaliana* seedlings was crosslinked using 1% formaldehyde for 30 seconds. The ChIP was performed with 0.1 µg of ab817 per µg of chromatin; incubated together for 16 hours at 4°C. The immunoprecipitated DNA was quantified by Real-Time PCR. The bottom panel indicates the positive (ab817) and negative controls (no antibody) at region B3.



IHC - Wholemout - Anti-RNA polymerase II CTD  
repeat YSPTSPS antibody [8WG16] - ChIP Grade  
(ab817)  
This image is courtesy of an anonymous Abreview

IHC - Wholemout of *Caenorhabditis elegans* embryo labelling RNA polymerase II CTD repeat YSPTSPS with ab817. Sample was incubated with primary antibody (1/100 in PBS + 3% BSA + 0.1% Triton-X 100) for 24 hours at 4°C. [ab150113](#), a goat [anti-mouse Alexa Fluor® 488](#) (undiluted) was used as the secondary antibody.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [8WG16] - ChIP Grade (ab817)

**All lanes :** Anti-RNA polymerase II CTD repeat YSPTSPS antibody [8WG16] - ChIP Grade (ab817) at 0.25 µg/ml

**Lane 1 :** HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate

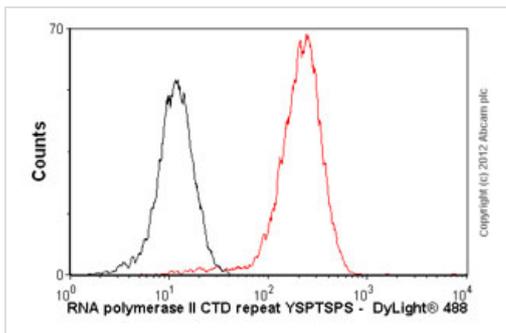
**Lane 2 :** THP-1 (human monocytic leukemia cell line) cell lysate

**Lane 3 :** RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate

Lysates/proteins at 15 µg per lane.

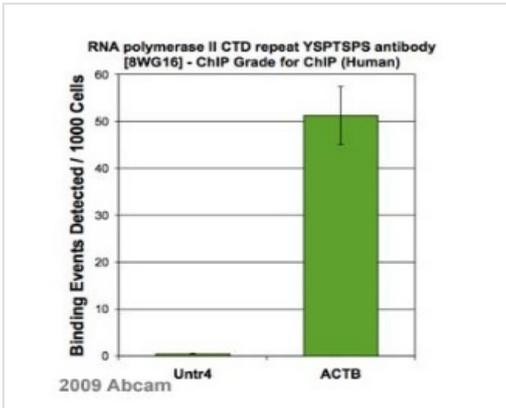
Developed using the ECL technique.

**Predicted band size:** 217 kDa



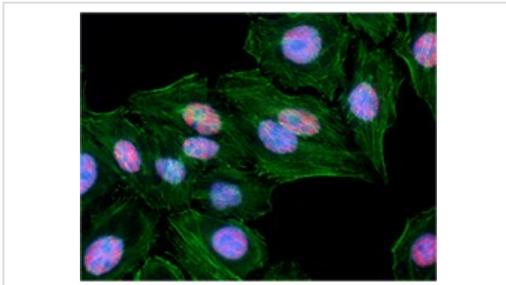
Flow Cytometry - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [8WG16] - ChIP Grade (ab817)

Overlay histogram showing HeLa cells stained with ab817 (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 (ab817, 0.5µg/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was a [Goat Anti-Mouse IgG H&L \(DyLight® 488\) preadsorbed \(ab96879\)](#) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] ([ab91361](#), 1µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed.



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [8WG16] - ChIP Grade (ab817)  
This image is a courtesy of Genpathway Inc

Chromatin was prepared from nuclear lysate of the human MCF7 breast epithelial adenocarcinoma cells. The cross-linking (X-ChIP) technique was used, crosslinking was performed for 15 minutes in formaldehyde. The primary antibody was used in concentration of 0.2 µg/µg chromatin and incubated with the sample for 16 hours at 4°C in SDS, DOC, TritonX-100, EDTA, HEPES, NaCl. The immunoprecipitated DNA was quantified by real time PCR. Ct values were converted to DNA copy numbers using a standard curve in the Q-PCR step. The number of binding events detected for each test reaction was then calculated by taking into account the DNA copy number, cell equivalents of chromatin used in the ChIP and PCR, and primer pair amplification efficiency.



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [8WG16] - ChIP Grade (ab817)

2% PFA-fixed, 0.5% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained for RNA polymerase II CTD repeat YSPTSPS using ab817 (red) at 2 µg/ml in ICC/IF. Secondary antibody: DyLight™ 594 conjugated goat anti-mouse IgG for 1 hour at RT. Actin filaments were labeled with Alexa Fluor® 488 Phalloidin (green). Nuclei were counterstained with DAPI (blue).

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