abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade ab26721

11 Abreviews 40 References 7 图像

概述

产品名称	Anti-RNA polymerase II CTD repeat YSPTSPS抗体- ChIP Grade
描述	兔多克隆抗体to RNA polymerase II CTD repeat YSPTSPS - ChIP Grade
宿主	Rabbit
特异性	Replenishment batches of our polyclonal antibody, ab26721 are tested in ChIP. Previous batches were additionally validated in ICC/IF, IHC-P, IP and WB. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody, **ab238146**.
经测试应用	**适用于:** WB, IHC-P, IP, ICC/IF, ChIP
种属反应性	**与反应:** Human **预测可用于:** Mouse, Pig, Schizosaccharomyces pombe
免疫原	Synthetic peptide corresponding to Human RNA polymerase II CTD repeat YSPTSPS conjugated to keyhole limpet haemocyanin. Database link: **P24928** (Peptide available as **ab17564**)
阳性对照	ICC/IF: HeLa cells IHP-P: Human Hodgkin lymphoma tissue WB: HeLa, HEK293, MEL and K562 cell lysates IP: HeLa
常规说明	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

性能

形式	Liquid
存放说明	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.

存储溶液	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.
纯度	Immunogen affinity purified
克隆	**多克隆**
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab26721于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★☆☆ (3)	Use a concentration of 1 µg/ml. Detects a band of approximately 220 kDa (predicted molecular weight: 220 kDa).
IHC-P	★★★★☆ (1)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use a concentration of 5 µg/ml.
ICC/IF	★★★★★ (2)	Use a concentration of 1 µg/ml.
ChIP	★★★★☆ (4)	Use 5-10 µg for 25 µg of chromatin.

靶标

功能 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.

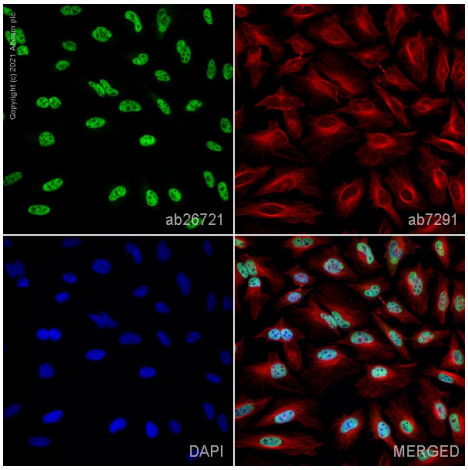
序列相似性 Belongs to the RNA polymerase beta' chain family.

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

翻译后修饰 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.

细胞定位 Nucleus.

图片

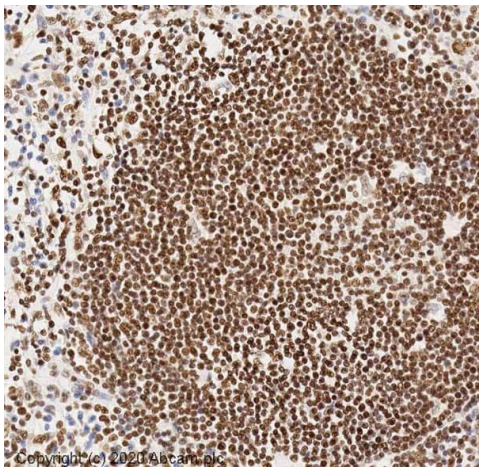


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade (ab26721)

ab26721 staining RNA polymerase II CTD repeat YSPTSPS in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 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 ab26721 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 **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

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

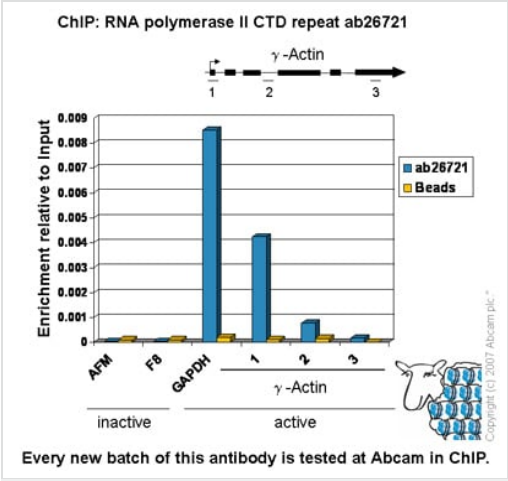


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade (ab26721)

IHC image of RNA polymerase II CTD repeat YSPTSPS staining in a section of formalin-fixed paraffin-embedded normal human Hodgkin's lymphoma* performed on a Leica BOND™ system using the standard protocol **F**. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab26721, 1ug/ml, for 15 mins 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.

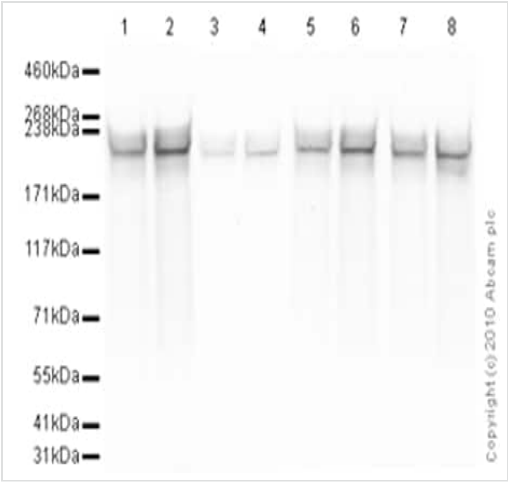
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.*

Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade (ab26721)

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 5µg of ab26721 (blue), and 20µl 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 g-Actin gene (active). Schematic diagram of the g-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.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade (ab26721)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade (ab26721) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 3 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human RNA polymerase II CTD repeat YSPTSPS peptide (**ab17564**) at 1 µg/ml
Lane 4 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate with Human RNA polymerase II CTD repeat YSPTSPS peptide (**ab17564**) at 1 µg/ml
Lane 5 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide (**ab18488**) at 1 µg/ml
Lane 6 : HEK293 (Human embryonic kidney cell line) 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) Whole Cell Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide (**ab12793**) at 1 µg/ml
Lane 8 : HEK293 (Human embryonic kidney cell line) 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

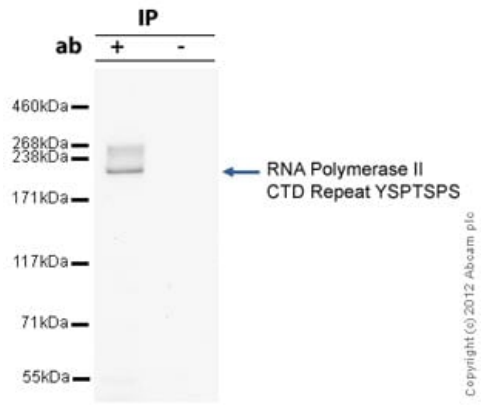
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) (**ab65484**) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

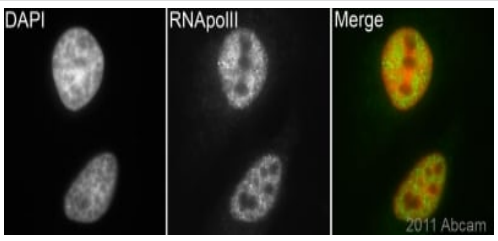
Predicted band size: 220 kDa
Observed band size: 220 kDa

Exposure time: 1 minute



Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade (ab26721)

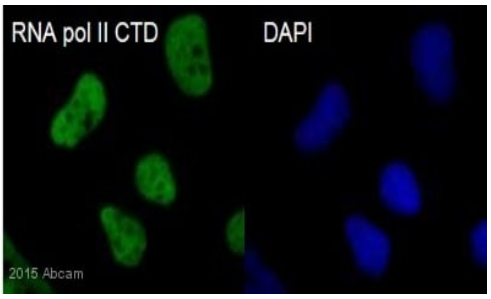
RNA polymerase II CTD repeat YSPTSPS was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to RNA polymerase II CTD repeat YSPTSPS and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab26721.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to mouse **anti-Rabbit HRP** (IgG light chain) (**ab99697**).
Band: 220kDa; RNA polymerase II CTD repeat YSPTSPS.



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade (ab26721)

Image courtesy of an AbReview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MICB, Canada

ab26721 (1/200) staining RNA polymerase II in assyncchonous HeLa cells (green). cells were fixed in paraformaldehyde, permeabilised in 0.5% Triton X100 and counterstained with DAPI in order to highlight the nucleus (red). For further experimental details please refer to Abreview.



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS antibody - ChIP Grade (ab26721)

This image is courtesy of an anonymous AbReview.

ab26721 staining RNA polymerase II CTD in the human HeLa cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with Tween-20 and blocked with 1% BSA for 10 minutes at 25°C. Samples were incubated with primary antibody (1/400 in TBS) for 12 hours at 4°C. A FITC conjugated anti-rabbit goat IgG polyclonal (1/500) was used as the secondary antibody.

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