abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] ab76292

Recombinant RabMAb

10 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y]
Description	Rabbit monoclonal [EP1510Y] to RNA polymerase II CTD repeat YSPTSPS (phospho S5)
Host species	Rabbit
Specificity	Detects RNA Polymeraste II phosphorylated at serine 5.
Tested applications	**Suitable for:** WB, IHC-P, Dot blot, ICC/IF **Unsuitable for:** Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human, Mouse, and Rat brain lysate. Dot Blot: RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide. IHC-P: Human colon, Human colon cancer, Mouse and Rat testis tissue. ICC/IF: HeLa cells.
General notes	This product has switched from a hybridoma to recombinant production method on 9th February 2024. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1510Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab76292 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
WB		1/10000. Predicted molecular weight: 217 kDa.
IHC-P		1/4000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Dot blot		1/1000.
ICC/IF		1/1000.

Application notes Is unsuitable for Flow Cyt.

Target

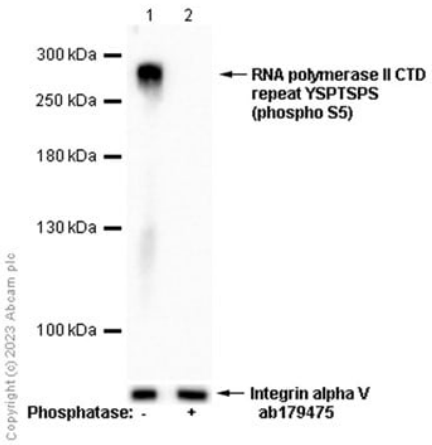
Function DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Largest and catalytic component of RNA polymerase II which synthesizes mRNA precursors and many functional non-coding RNAs. Forms the polymerase active center together 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



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292) at 1/10000 dilution

Lane 1 : Human brain lysate
Lane 2 : Human brain lysate, then the membrane treated with Alkaline Phosphatase for 1 hour

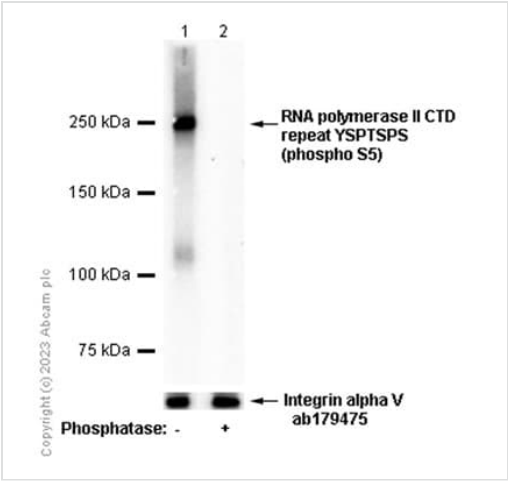
Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 217 kDa
Observed band size: 270 kDa

Exposure time: 40 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292) at 1/10000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse brain lysate, then the membrane treated with Alkaline Phosphatase for 1 hour

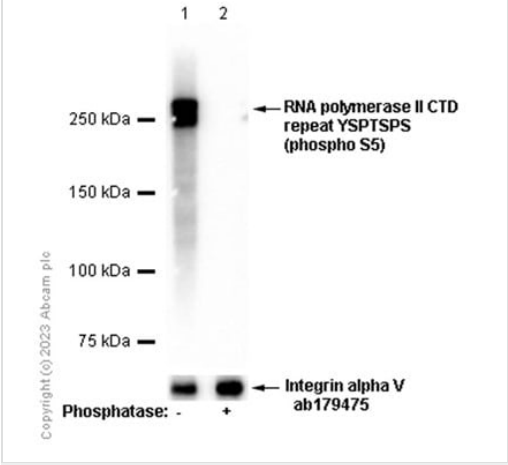
Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 217 kDa
Observed band size: 270 kDa

Exposure time: 60 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292) at 1/10000 dilution

Lane 1 : Rat brain lysate
Lane 2 : Rat brain lysate, then the membrane treated with Alkaline Phosphatase for 1 hour

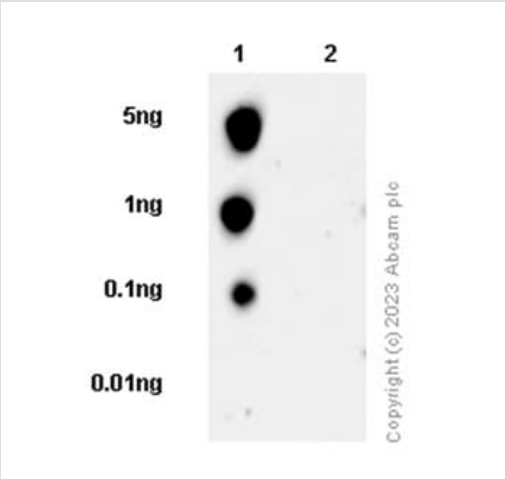
Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 217 kDa
Observed band size: 270 kDa

Exposure time: 20 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

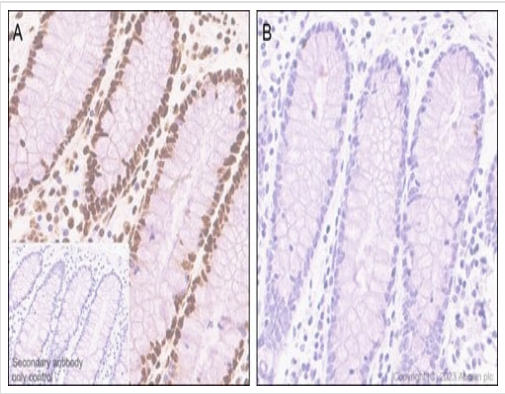


Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

Dot blot analysis of RNA polymerase II CTD repeat YSPTSPS (phospho S5) using ab76292 at 1/1000 dilution, followed by a Goat Anti-Rabbit IgG (H+L) Peroxidase conjugated (**ab97051**) at 1/2500 dilution. Blocking and dilution buffer: 5% NFDM/TBST. Exposure time: 180s.

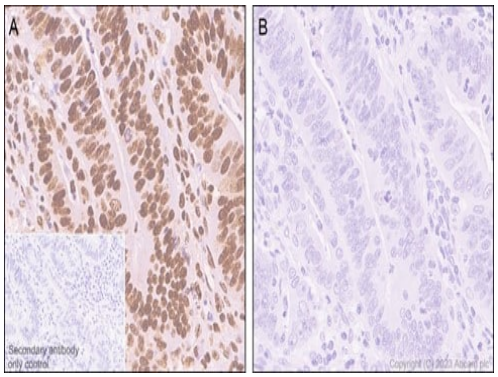
Lane 1: RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide

Lane 2: RNA polymerase II CTD repeat YSPTSPS non-phospho peptide



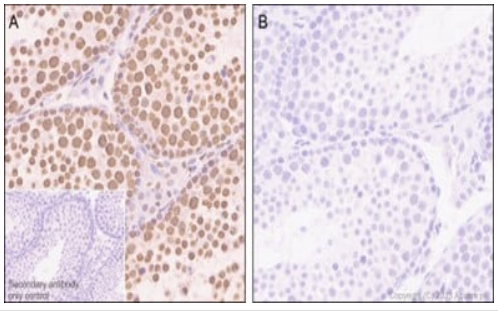
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab76292 at 1/4000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human colon without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



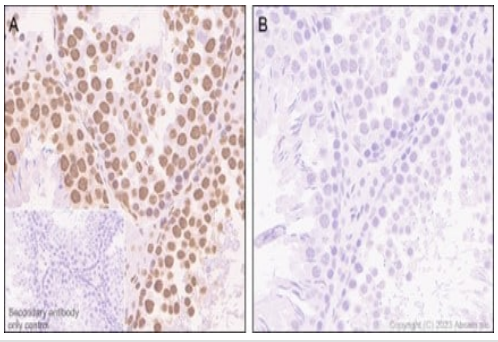
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

Immunohistochemical analysis of paraffin-embedded Human colon cancer tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab76292 at 1/4000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human colon cancer without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



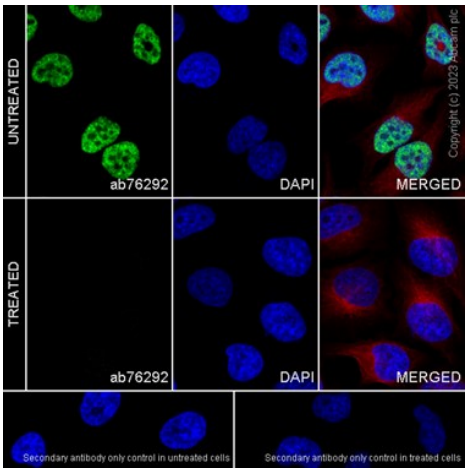
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab76292 at 1/4000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on mouse testis without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



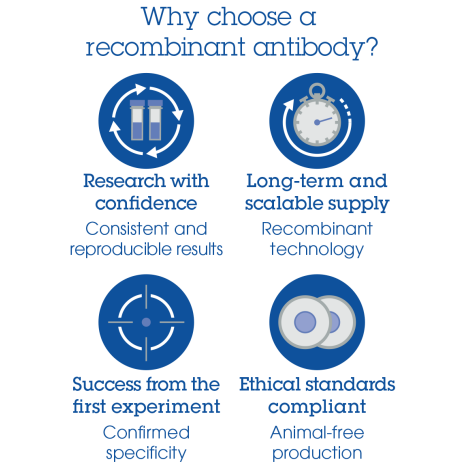
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab76292 at 1/4000 dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on rat testis without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling RNA polymerase II CTD repeat YSPTSPS with ab76292 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150081**) at 1/1000 dilution. Alexa Fluor® 594 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (**ab195889**) was used to counterstain tubulin at 1/200 dilution. The nuclear counterstain was DAPI (Blue).



Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EP1510Y] (ab76292)

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