abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade ab238146

Recombinant RabMAb

2 Abreviews 16 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade
Description	Rabbit monoclonal [EPR18855-87] to RNA polymerase II CTD repeat YSPTSPS (phospho S2) - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** ChIP-sequencing, WB, Dot blot, IHC-P, ICC/IF, ChIP, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, RAW 264.7 and PC-12 whole cell lysates. Dot blot: RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide. IHC-P: Mouse testis tissue; rat testis tissue; human testis tissue. ICC/IF: HeLa and RAW 264.7 cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from HeLa cells.
General notes	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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18855-87
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab238146 in the following tested applications.

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

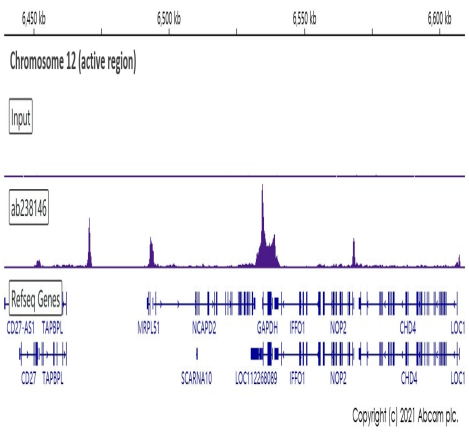
Application	Abreviews	Notes
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
WB		1/2000. Detects a band of approximately 270 kDa (predicted molecular weight: 217 kDa).
Dot blot		1/1000.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
ChIP	★★☆☆☆ (1)	Use 5 µg for 25 µg of chromatin.
IP		1/30.
Flow Cyt (Intra)		1/600.

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.

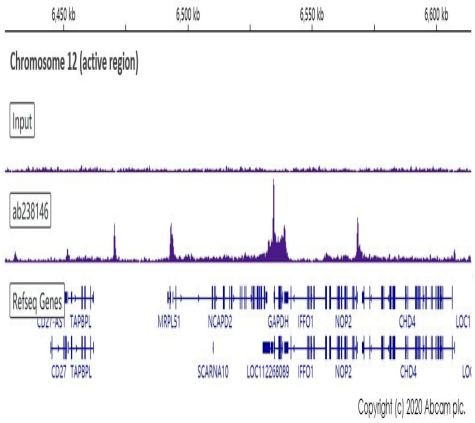
Images



ChIP-sequencing - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 µg of chromatin and 4 µg of ab238146. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. ChIP-Seq validation performed with ChIP-Kit Transcription Factors ChIP-Seq (**ab270813**).

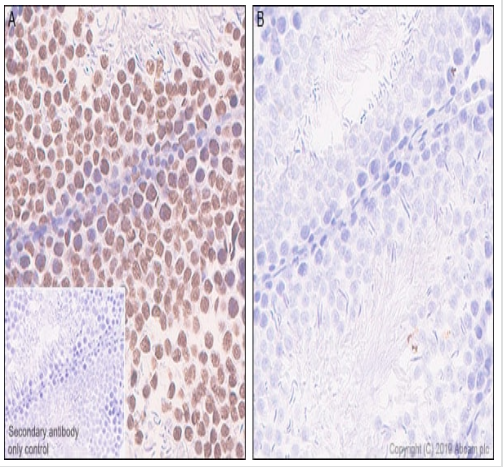
Additional screenshots of mapped reads can be downloaded **here**.



ChIP-sequencing - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 cells and 4 µg of Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146). ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

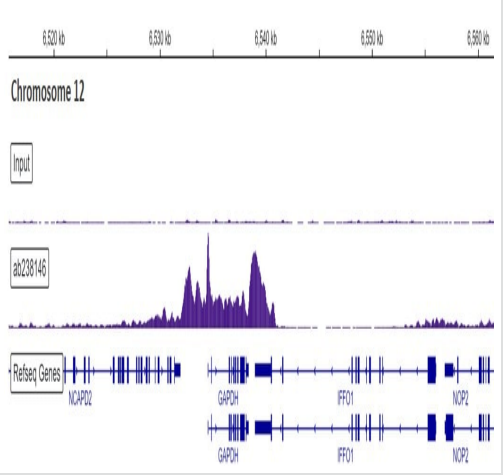
Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/2000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on rat testis without alkaline phosphatase treatment (panel A). No signal was detected when treated with alkaline phosphatase (panel B). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab238146 for 30 mins at room temperature.

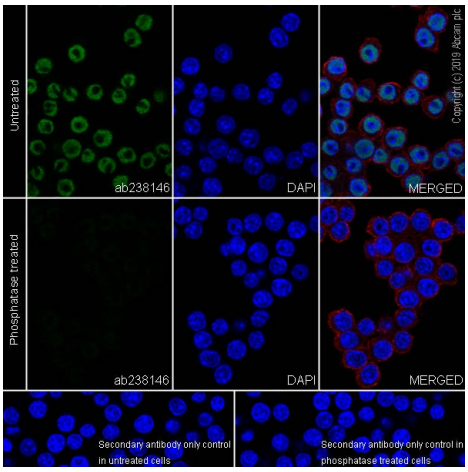
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



ChIP-sequencing - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 µg of chromatin and 4 µg of Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146). ChIP DNA was sequenced on the Illumina NextSeq 500 to a depth of 30 million reads. ChIP-Seq validation performed by Active Motif, Carlsbad, CA.

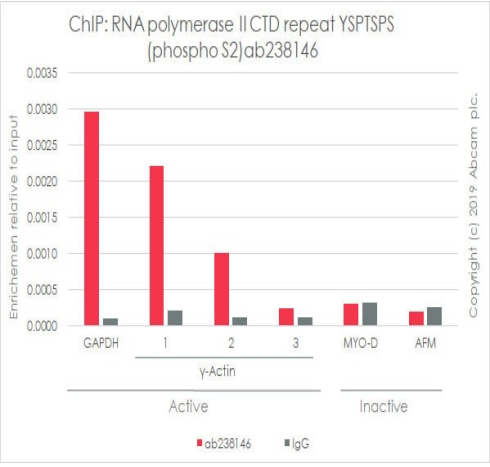
Additional screenshots of mapped reads can be downloaded **here**.



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/500 dilution, followed by a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in RAW 264.7 cell line, the signal decreased after phosphatase treatment at 37▌ for 2h. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

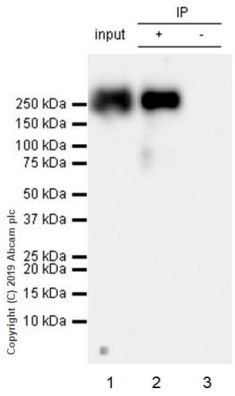


ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

Chromatin was prepared from HeLa (human epithelial cell line from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol. Cells were fixed with 1% formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab238146 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (sybr green approach).

Primers and probes are located in the first kb of the transcribed region.

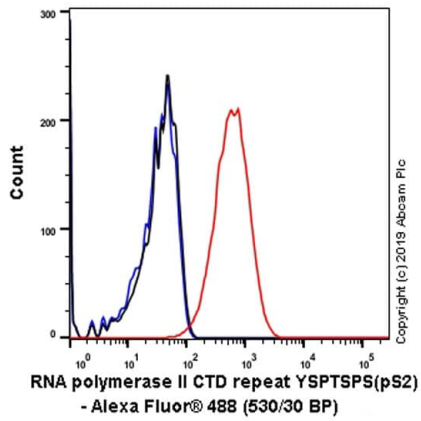


Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

RNA polymerase II CTD repeat YSPTSPS (phospho S2) was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab238146 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab238146 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used as secondary antibody at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (Input).
Lane 2: **ab237146** IP in HeLa whole cell lysate.
Lane 3: : Rabbit monoclonal IgG (**ab172730**) instead of ab238146 in HeLa whole cell lysate.

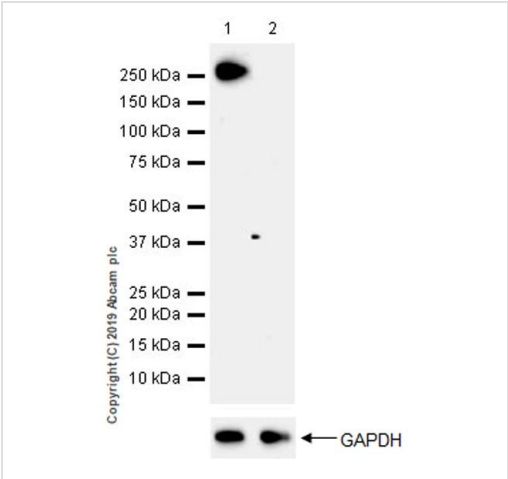
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 7 seconds.



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

Intracellular flow cytometric analysis of4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/600 (red) compared with a Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue).

Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**), at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146) at 1/5000 dilution

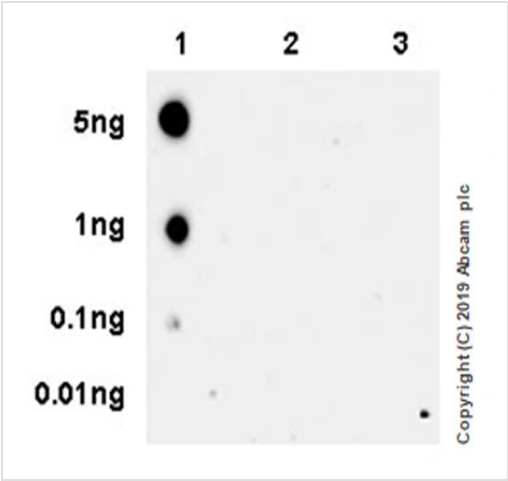
Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa whole cell lysate (phosphatase-treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 3 minutes



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

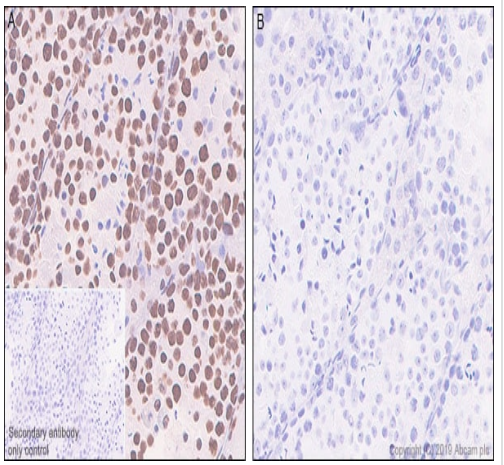
Dot blot analysis of RNA polymerase II CTD repeat YSPTSPS (phospho S2) labeled with ab238146 at 1/1000 dilution.

Lane 1: RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide.
Lane 2: RNA polymerase II CTD repeat YSPTSPS non-phospho peptide.
Lane 3: RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide.

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

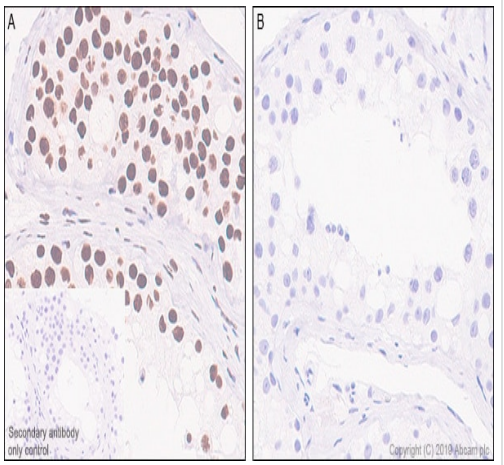
Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/2000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on mouse testis without alkaline phosphatase treatment (panel A). No signal was detected when treated with alkaline phosphatase (panel B). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab238146 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/2000 dilution, followed by a Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human testis without alkaline phosphatase treatment (panel A). No signal was detected when treated with alkaline phosphatase (panel B). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

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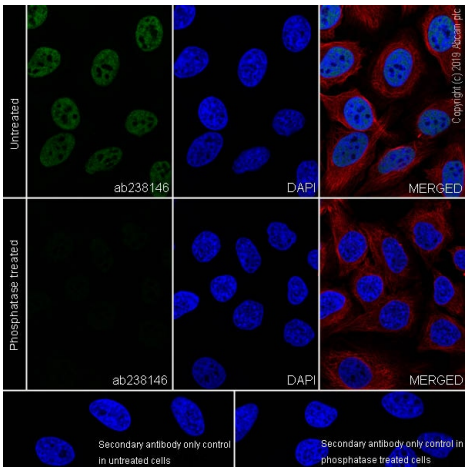
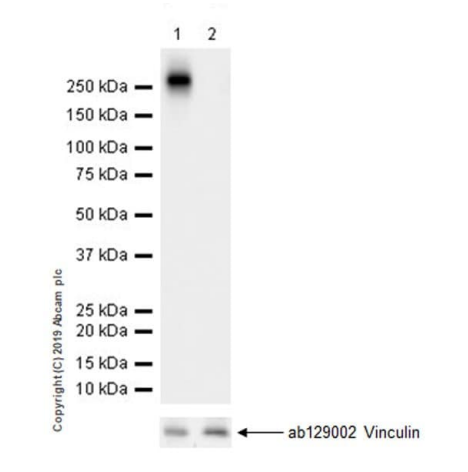


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/500 dilution, followed by a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HeLa cell line, the signal decreased after phosphatase treatment at 37I for 2h. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146) at 1/2000 dilution

Lane 1 : RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 2 : RAW 264.7 whole cell lysate (phosphatase-treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

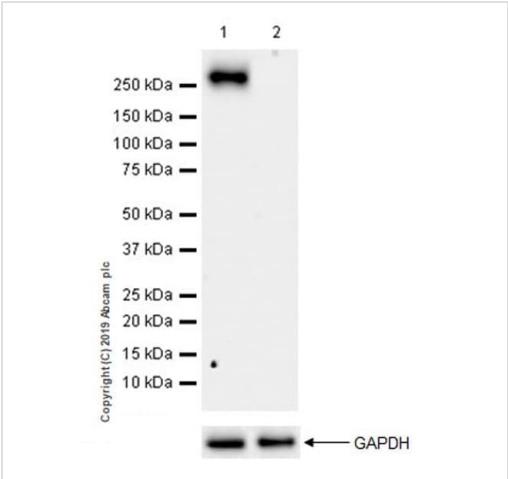
Predicted band size: 217 kDa
Observed band size: 270 kDa

Exposure time: 37 seconds

Blocking and dilution buffer: 1% BSA/TBST.

The molecular weight observed is consistent with what has been

described in the literature (PMID: 25849141).



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146) at 1/2000 dilution

Lane 1 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 2 : PC-12 whole cell lysate (phosphatase-treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 37 seconds

Blocking and dilution buffer: 1% BSA/TBST.

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - ChIP Grade (ab238146)

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