abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] ab193468

重组 RabMAb

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概述

产品名称	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2)**抗体**[EPR18855]
描述	**兔单克隆抗体**[EPR18855] to RNA polymerase II CTD repeat YSPTSPS (phospho S2)
宿主	Rabbit
经测试应用	**适用于:** WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra), Dot blot, ChIP
种属反应性	**与反应:** Mouse, Rat, Human **预测可用于:** Saccharomyces cerevisiae
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: MCF7, HeLa, RAW 264.7 and PC-12 whole cell lysates. IHC-P: Mouse kidney, spleen and testis tissues. ICC/IF: HeLa, MCF7 and PC-12 cells. IP: HeLa whole cell lysate. ChIP: HeLa cells.
常规说明	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**.

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 long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	EPR18855
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab193468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		1/5000. Detects a band of approximately 270 kDa (predicted molecular weight: 192 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Recommended for mouse only.
ICC/IF	★★★★★ (1)	1/100.
IP		1/50.
Flow Cyt (Intra)		Use at an assay dependent concentration.
Dot blot		1/1000.
ChIP		Use at an assay dependent concentration.

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.

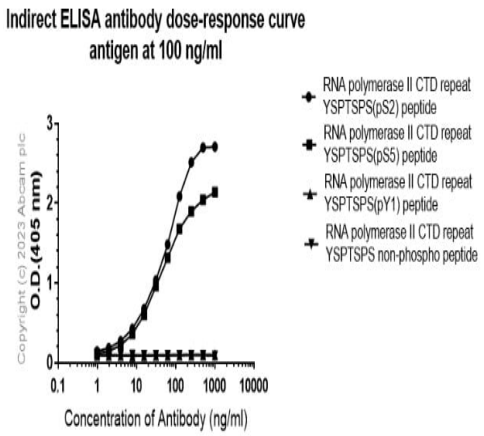
翻译后修饰

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



Indirect ELISA - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Indirect ELISA using ab193468 and secondary antibody Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) at 1/2500 dilution.

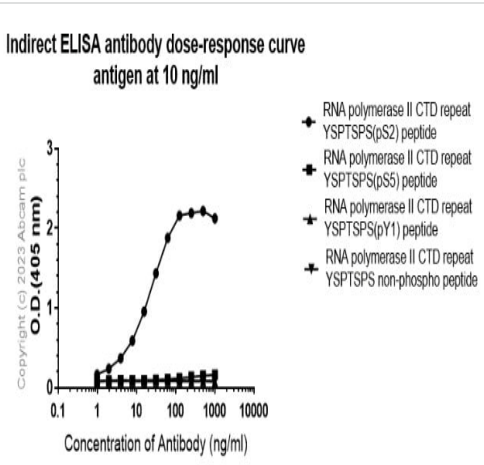
Antigens were at 100ng/mL concentration

RNA polymerase II CTD repeat YSPTSPS(pS2) peptide,

RNA polymerase II CTD repeat YSPTSPS(pS5) peptide,

RNA polymerase II CTD repeat YSPTSPS(pY1) peptide,

RNA polymerase II CTD repeat YSPTSPS non-phospho peptide



Indirect ELISA - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Indirect ELISA using ab193468 and secondary antibody Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) at 1/2500 dilution.

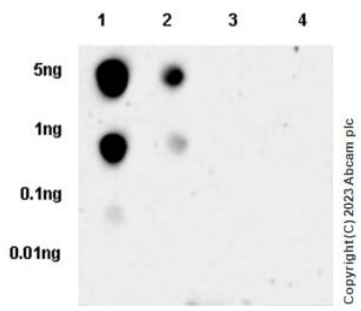
Antigens were at 10ng/mL concentration

RNA polymerase II CTD repeat YSPTSPS(pS2) peptide,

RNA polymerase II CTD repeat YSPTSPS(pS5) peptide,

RNA polymerase II CTD repeat YSPTSPS(pY1) peptide,

RNA polymerase II CTD repeat YSPTSPS non-phospho peptide



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Labeled using ab193468 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) secondary antibody (**ab97051**) at 1/100000 dilution.

Blocking/Dilution buffer: 5% NFDM/TBST.

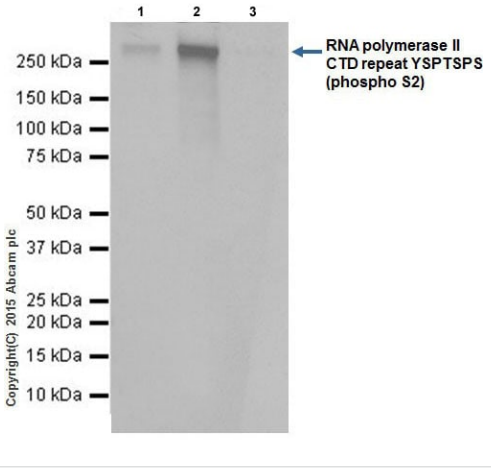
Exposure time: 3 minutes.

Lane 1: RNA polymerase II CTD repeat YSPTSPS (pS2) phospho peptide

Lane 2: RNA polymerase II CTD repeat YSPTSPS (pS5) phospho peptide

Lane 3: RNA polymerase II CTD repeat YSPTSPS (pY2) phospho peptide

Lane 4: RNA polymerase II CTD repeat YSPTSPS non-phospho peptide



Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

RNA polymerase II CTD repeat YSPTSPS (phospho S2) was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab193468 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab193468 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

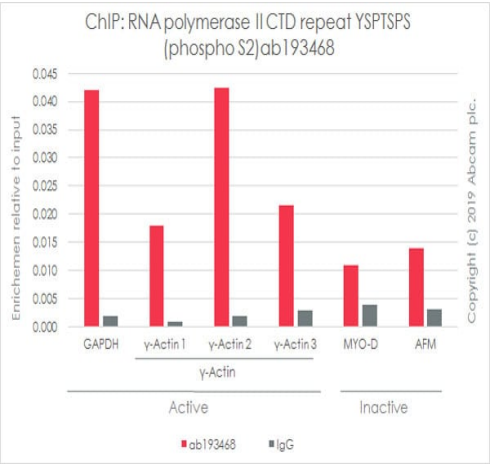
Lane 1: HeLa whole cell lysate 10µg (Input).

Lane 2: ab193468 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab193468 in HeLa whole cell lysate.

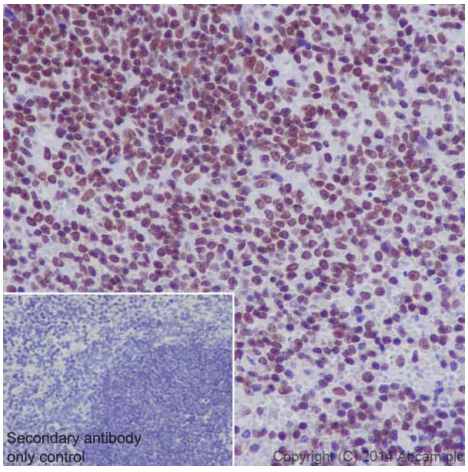
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Chromatin was prepared from HeLa cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab193468 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µL of Protein A/G sepharose beads. 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.
*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol

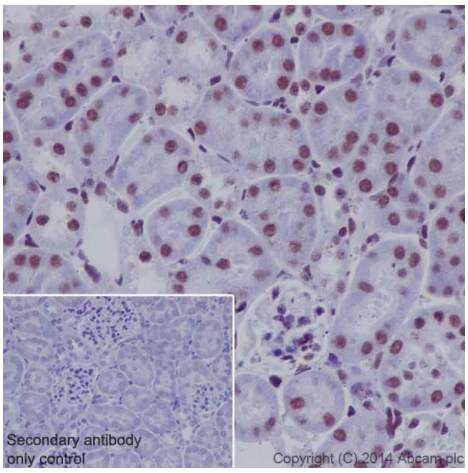


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab193468 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on mouse spleen is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

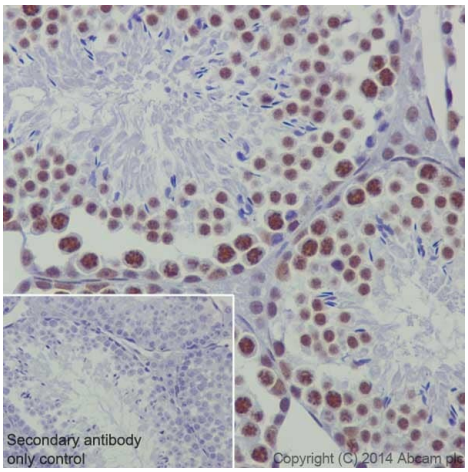


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab193468 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on epithelium cells and glomerulus cells of mouse kidney is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

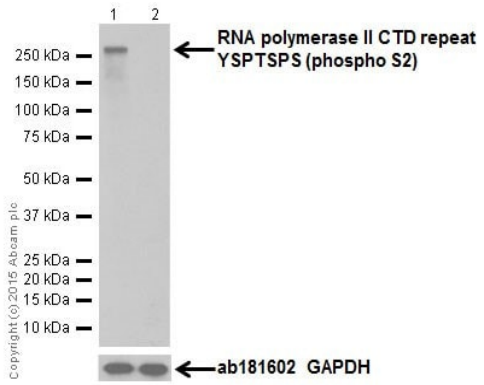


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab193468 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on mouse testis is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468) at 1/5000 dilution

Lane 1 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate
Lane 2 : MCF7 whole cell lysate treated with Lambda Phosphatase

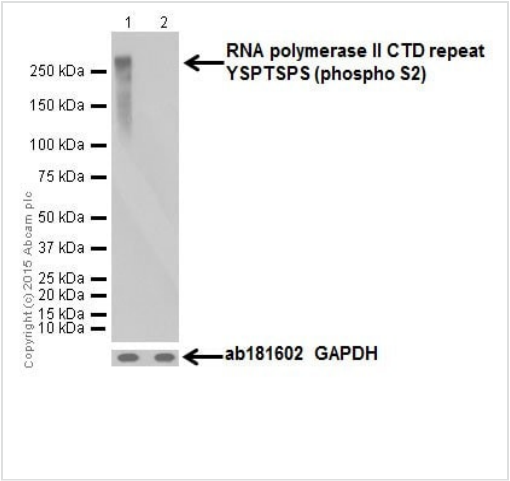
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 192 kDa
Observed band size: 270 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 1% BSA/TBST.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468) at 1/5000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa whole cell lysate treated with Lambda Phosphatase

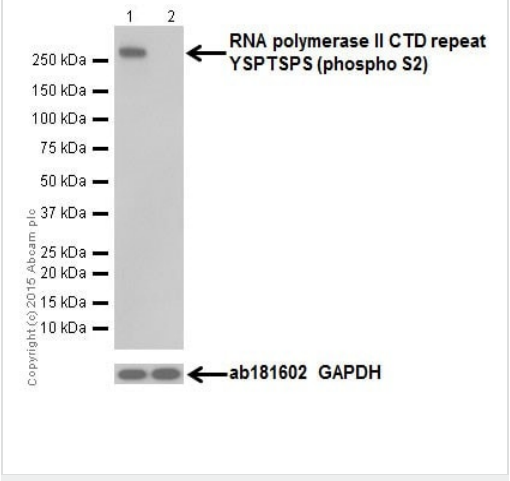
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 192 kDa
Observed band size: 270 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 1% BSA/TBST.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468) at 1/5000 dilution

Lane 1 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate
Lane 2 : RAW 264.7 whole cell lysate treated with Lambda Phosphatase

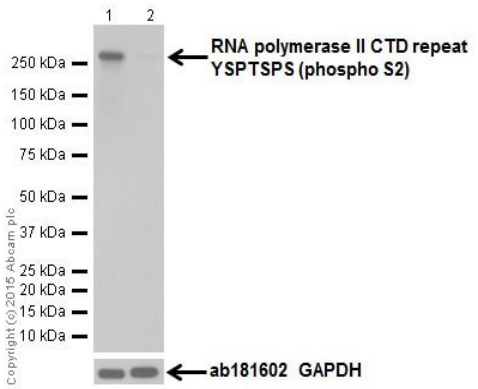
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 192 kDa
Observed band size: 270 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 1% BSA/TBST.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468) at 1/5000 dilution

Lane 1 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 2 : PC-12 whole cell lysate treated with Lambda Phosphatase

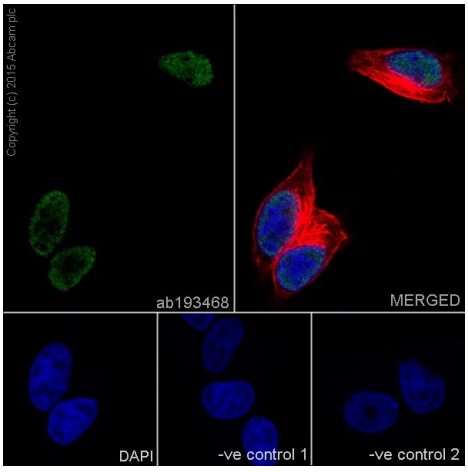
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 192 kDa
Observed band size: 270 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 1% BSA/TBST.



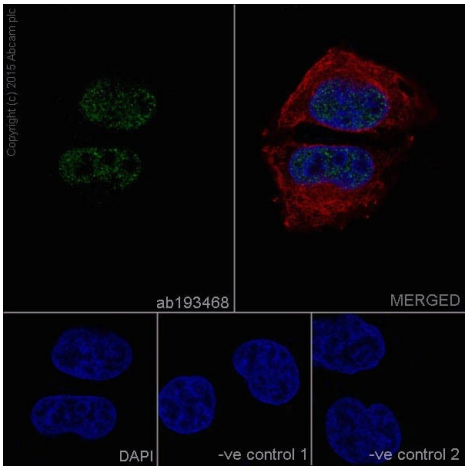
Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF7 (Human breast adenocarcinoma cell line) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab193468 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on MCF7 cell line. The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab193468 at 1/100 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.

-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



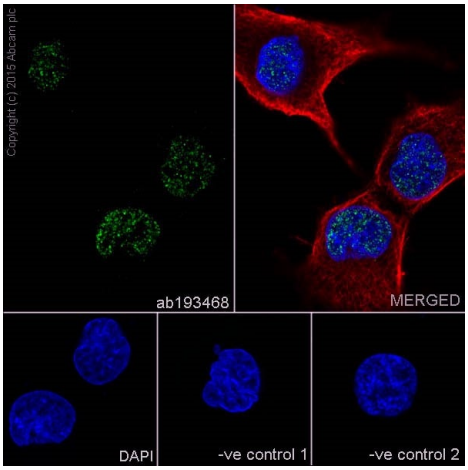
Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab193468 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab193468 at 1/100 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.

-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



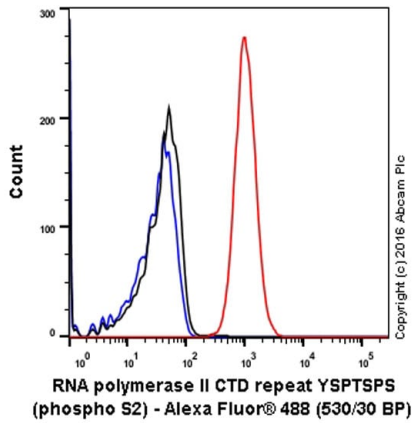
Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized PC-12 (Rat adrenal gland pheochromocytoma) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab193468 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on PC-12 cell line. The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

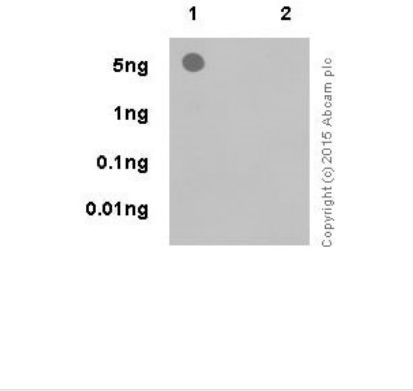
-ve control 1: ab193468 at 1/100 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.

-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling RNA polymerase II CTD repeat YSPTSPS antibody (phospho S2) (red) with purified ab193468 at a dilution of 1/70. Goat anti rabbit IgG (Alexa Fluor® 488) was used as the secondary antibody at 1/2000. Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Isotype control antibody was Rabbit monoclonal IgG (black). The blue line shows cells without incubation with primary antibody and secondary antibody.

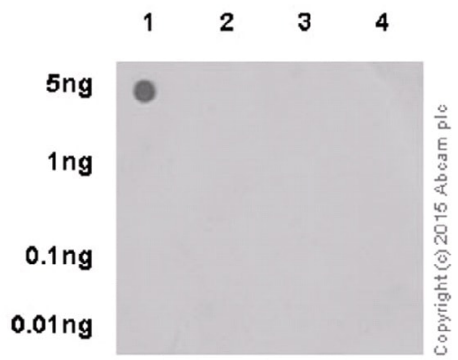


Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Dot blot analysis of RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide (Lane 1) and non-phospho peptide (Lane 2) labeled using ab193468 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated secondary antibody (**ab97051**) at 1/100000 dilution.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



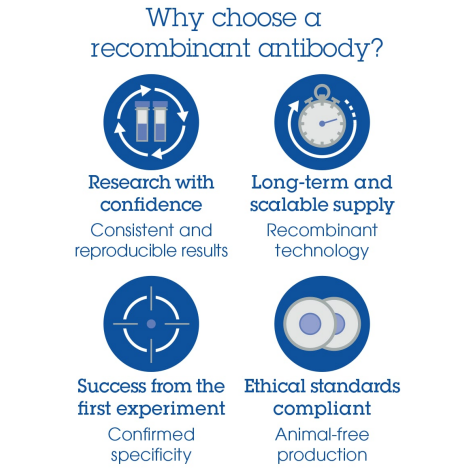
Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Lane 1: RNA polymerase II CTD repeat YSPTSPS (phospho S2) phospho peptide
Lane 2: RNA polymerase II CTD repeat YSPTSPS non-phospho peptide (peptide of ab193468)
Lane 3: RNA polymerase II CTD repeat YSPTSPS (phospho S5) phospho peptide
Lane 4: RNA polymerase II CTD repeat YSPTSPS non-phospho peptide (peptide of **ab193467**)

Labeled using ab193468 at 1/1000 dilution (0.8 ug/ml), followed by Goat Anti-Rabbit IgG H&L (HRP) secondary antibody (**ab97051**) at 1/100000 dilution.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855] (ab193468)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery

- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

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