abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] ab193467

Recombinant RabMAb

2 References 13 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015]
Description	Rabbit monoclonal [EPR19015] to RNA polymerase II CTD repeat YSPTSPS (phospho S5)
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, IP, ICC/IF, WB, Dot blot
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, PC-12 whole cell lysates. IHC-P: Mouse kidney and spleen tissues. ICC/IF: HeLa, RAW 264.7 and PC-12 cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate. Dot blot: RNA polymerase II CTD repeat YSPTSPS (phospho S5) phospho peptide.
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	EPR19015
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab193467 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
Flow Cyt (Intra)		1/30. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/20.
ICC/IF		1/250.
WB		1/1000. Detects a band of approximately 270 kDa (predicted molecular weight: 192 kDa).
Dot blot		1/1000.

Target

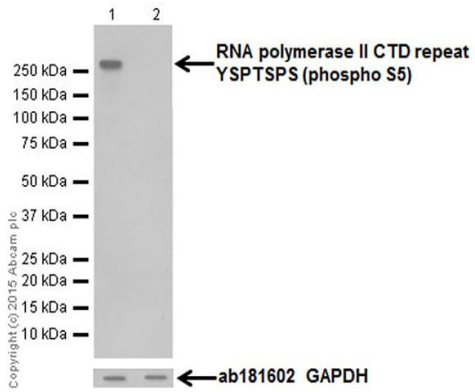
Function DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Largest and catalytic component of RNA polymerase II which synthesizes mRNA precursors and many functional non-coding RNAs. Forms the polymerase active center together 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 [EPR19015] (ab193467)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467) at 1/5000 dilution

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

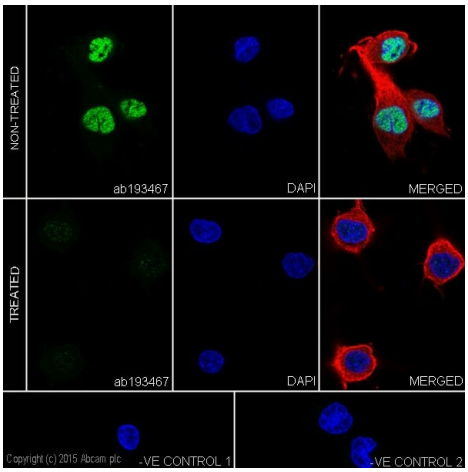
Lysates/proteins at 10 µg per lane.

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: 5 seconds

Blocking/Dilution buffer: 2% BSA/TBST.



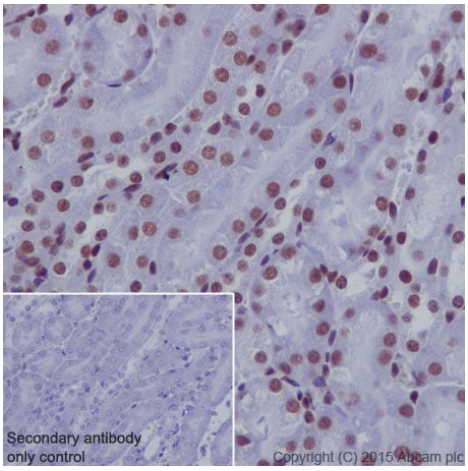
Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized PC-12 (Rat adrenal gland pheochromocytoma cell line) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S5) with ab193467 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on PC-12 cell line. The expression decreased after treatment with Lambda Phosphatase at 31°C for 5h.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab193467 at 1/250 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

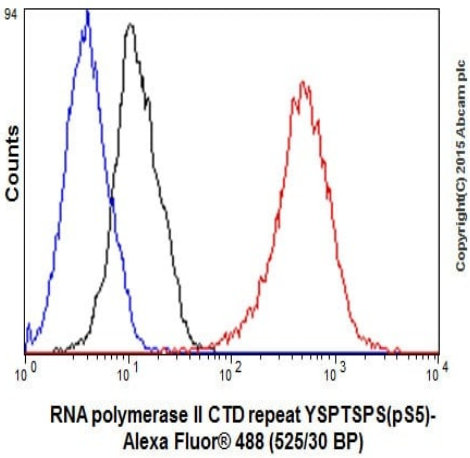


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S5) with ab193467 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on epithelial cells of mouse kidney is observed. Counter stained with Hematoxylin.

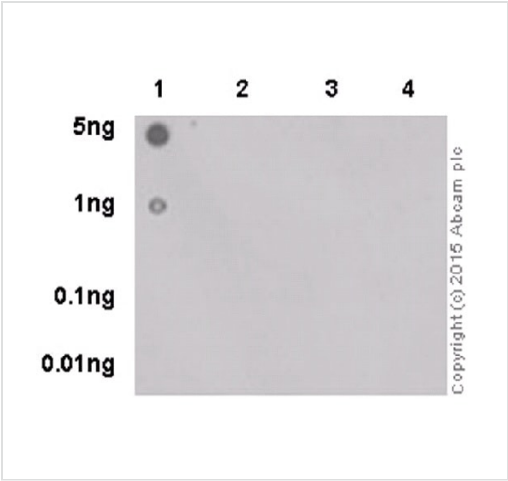
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S5) with ab193467 at 1/30 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



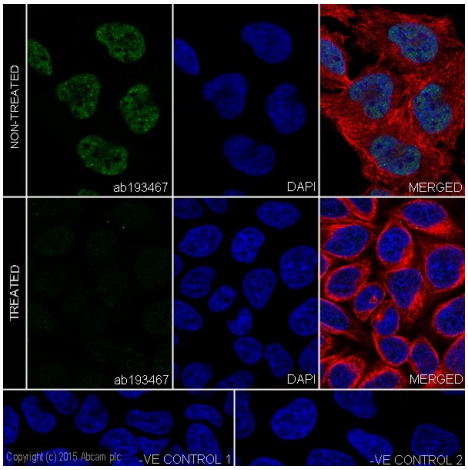
Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

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

Labeled using ab193467 at 1/1000 dilution (0.1 µg/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.



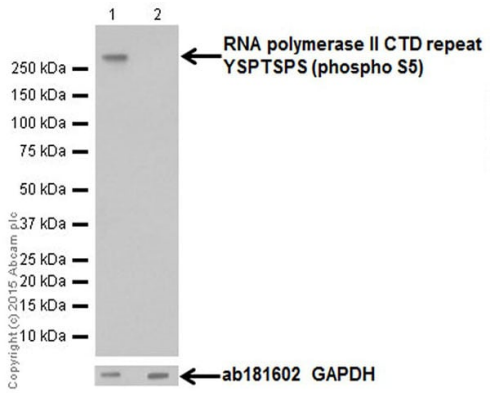
Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

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 S5) with ab193467 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on HeLa cell line. The expression decreased after treatment with Lambda Phosphatase 31°C for 5h.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab193467 at 1/250 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution 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 S5) antibody [EPR19015] (ab193467)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467) at 1/1000 dilution

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

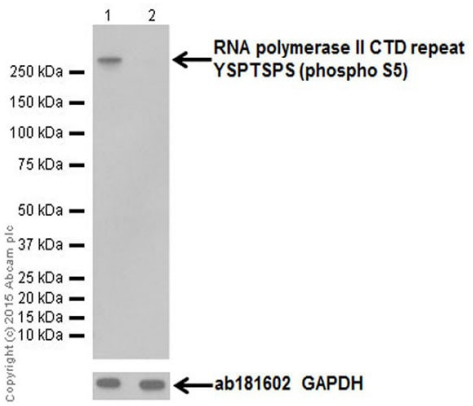
Lysates/proteins at 10 µg per lane.

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: 5 seconds

Blocking/Dilution buffer: 2% BSA/TBST.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467) at 1/1000 dilution

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

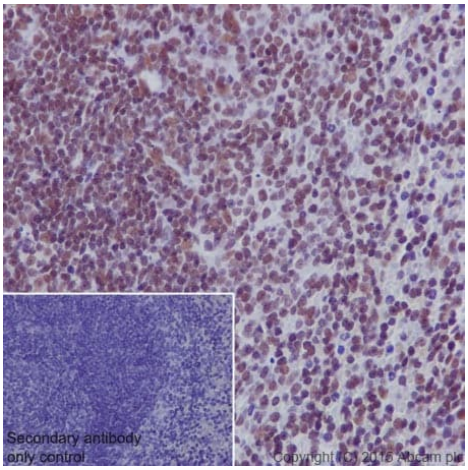
Lysates/proteins at 10 µg per lane.

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: 1 second

Blocking/Dilution buffer: 2%BSA/TBST.

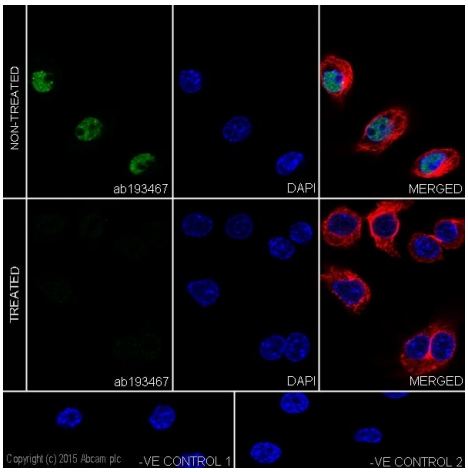


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S5) with ab193467 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on lymphocytes of 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.



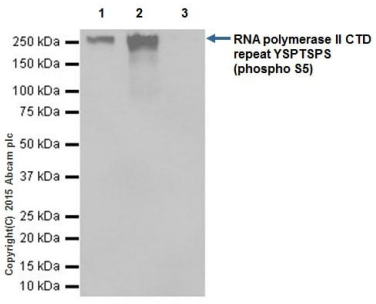
Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

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 S5) with ab193467 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on RAW 264.7 cell line. The expression decreased after treatment with Lambda Phosphatase 31°C for 5h.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab193467 at 1/250 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

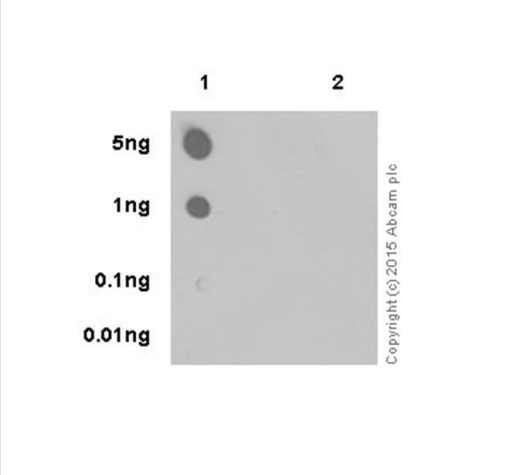


Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) was immunoprecipitated from 1mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab193467 at 1/20 dilution. Western blot was performed from the immunoprecipitate using ab193467 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: ab193467 IP in HeLa whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab193467 in HeLa whole cell lysate.

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

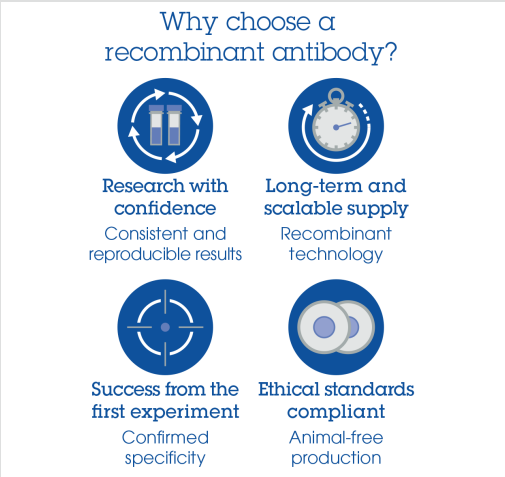


Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

Dot blot analysis of RNA polymerase II CTD repeat YSPTSPS (phospho S5) phospho peptide (Lane 1) and RNA polymerase II CTD repeat YSPTSPS non-phospho peptide (Lane 2) labeled using ab193467 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) secondary antibody (**ab97051**) at 1/1000 dilution.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [EPR19015] (ab193467)

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