abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free ab255849

Recombinant

8 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free
Description	Rat monoclonal [3E10] to RNA polymerase II CTD repeat YSPTSPS (phospho S2) - BSA and Azide free
Host species	Rat
Tested applications	**Suitable for:** Dot blot, ICC/IF, ChIP, ELISA, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Other Immunogen Type. This information is considered to be commercially sensitive.
Positive control	WB: HeLa, RAW264.7,and PC-12 whole cell lysates. ICC/IF: HeLa and RAW 264.7 cells.
General notes	ab255849 is the carrier-free version of ab252855. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. ab255849 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* 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. Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	3E10
Isotype	IgG1

Applications

Our Abpromise guarantee covers the use of **ab255849** 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
Dot blot		Use a concentration of 0.446 µg/ml.
ICC/IF		Use a concentration of 0.892 µg/ml.
ChIP		Use a concentration of 89.2 µg/ml.
ELISA		Use at an assay dependent concentration.
WB		Use a concentration of 0.446 µg/ml. Predicted molecular weight: 217 kDa.

Target

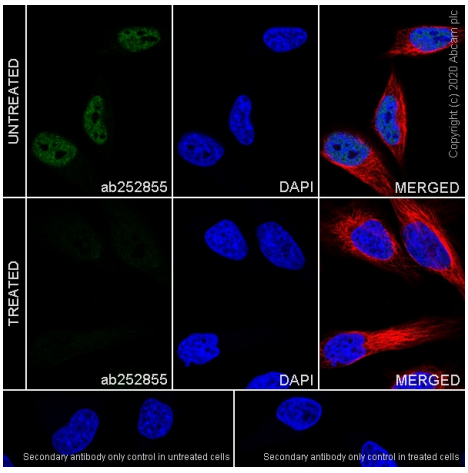
Target

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

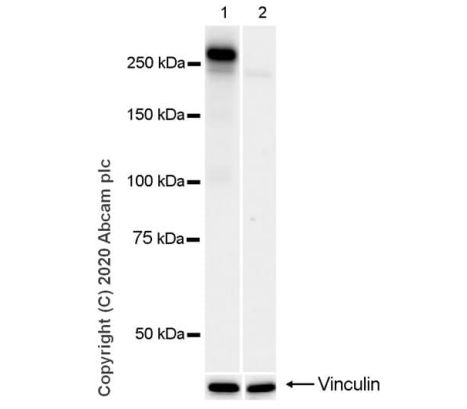


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free (ab255849)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab252855 at 1/500 dilution, followed by ab150113 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in HeLa cell line, the signal decreased after phosphatase treatment at 37ºC for 2h. ab179513 Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/500 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150113 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab252855).



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free (ab255849)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] (ab252855) at 1/1000 dilution

Lane 1 : PC-12 (rat adrenal gland pheochromocytoma), 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-Rat IgG (H+L), HRP) (ab205720) at 1/5000 dilution

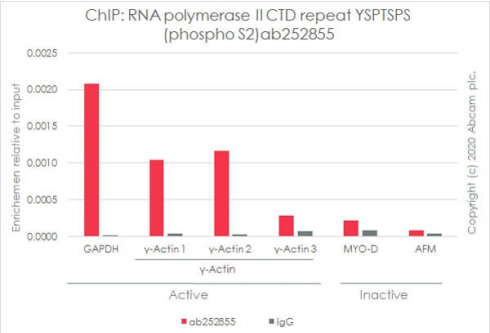
Predicted band size: 217 kDa
Observed band size: 260 kDa
why is the actual band size different from the predicted?

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

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 22745433 and 23071310)

Exposure time: 3 seconds

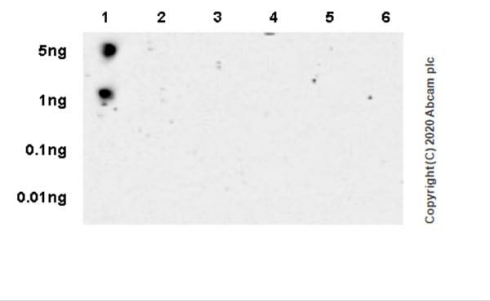
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab252855).



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free (ab255849)

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 ab252855 (red), or 5 µg of Rat IgG1 ab18407 (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).
http://www.abcam.com/resources?keywords=X%20ChIP%20protocol

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab252855).



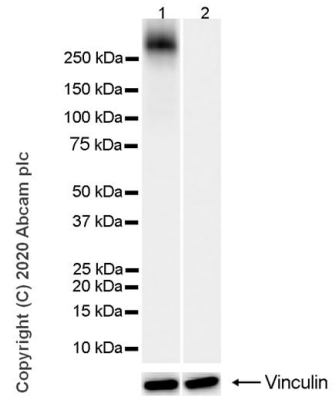
Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free (ab255849)

Dot blot analysis using ab252855 at 1/1000 dilution followed by a Goat Anti-Rat IgG (H+L), HRP) (ab205720) at 1/5000 dilution. Blocking/diluting buffer and concentration: 5% NFDM/TBST. Exposure time: 3 minutes.

Lane 1: RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide
Lane 2: RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide
Lane 3: RNA polymerase II CTD repeat YSPTSPS (phospho T4) peptide
Lane 4: RNA polymerase II CTD repeat YSPTSPS (phospho Y1) peptide
Lane 5: RNA polymerase II CTD repeat YSPTSPS (phospho S7) peptide
Lane 6: RNA polymerase II CTD repeat YSPTSPS non-phopho peptide

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (ab252855).



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free (ab255849)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] (ab252855) at 1/1000 dilution

Lane 1 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate (Untreated membrane)
Lane 2 : RAW264.7 whole cell lysate (Phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rat IgG (H+L), HRP) (ab205720) at 1/5000 dilution

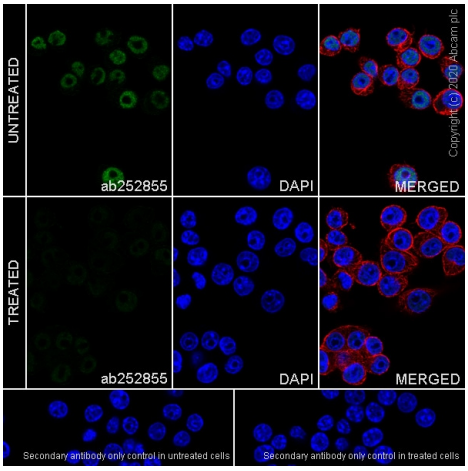
Predicted band size: 217 kDa
Observed band size: 260 kDa why is the actual band size different from the predicted?

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

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 22745433 and 23071310)

Exposure time: 7 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab252855).

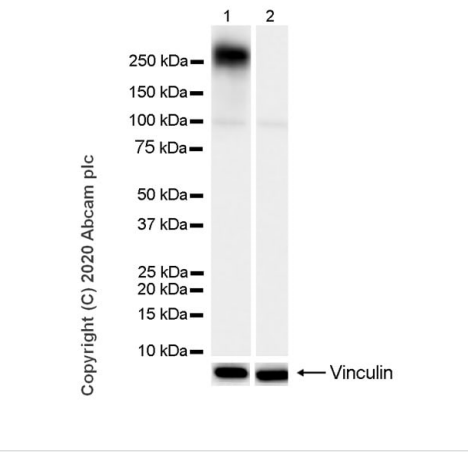


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free (ab255849)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 cells labelling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab252855 at 1/500 dilution, followed by ab150113 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in RAW 264.7 cell line, the signal decreased after phosphatase treatment at 37ºC for 2h. ab179513 Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/500 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150113 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab252855).



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free (ab255849)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] (ab252855) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate (Untreated membrane)
Lane 2 : HeLa whole cell lysate (Phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rat IgG (H+L), HRP) (ab205720) at 1/5000 dilution

Predicted band size: 217 kDa
Observed band size: 260 kDa why is the actual band size different from the predicted?

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

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 22745433 and 23071310)

Exposure time: 3 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab252855).

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [3E10] - BSA and Azide free (ab255849)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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