abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] - ChIP Grade ab252852

リコンビナント

3 References 画像数 9

製品の概要

製品名	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] - ChIP Grade
製品の詳細	Rat monoclonal [3E8] to RNA polymerase II CTD repeat YSPTSPS (phospho S5) - ChIP Grade
由来種	Rat
アプリケーション	**適用あり:** ICC/IF, WB, ChIP, Dot blot, Indirect ELISA **適用なし:** IP
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HeLa, RAW 264.7 and PC-12 whole cell lysates. ICC/IF: HeLa and RAW264.7 cells. ChIP: Chromatin prepared from HeLa cells.
特記事項	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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**.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
精製度	Ion Exchange Chromatography
ポリ/モノ	モノクローナル

クローン名	3E8
アイソタイプ	IgG2a

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab252852の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF		Use a concentration of 1.29 µg/ml.
WB		Use a concentration of 0.645 µg/ml. Detects a band of approximately 260 kDa (predicted molecular weight: 217 kDa).
ChIP		Use 5 µg for 25 µg of chromatin.
Dot blot		Use a concentration of 0.645 µg/ml.
Indirect ELISA		Use at an assay dependent concentration. Use at 250 ng/ml.

追加情報 Is unsuitable for IP.

ターゲット情報

機能 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.

配列類似性 Belongs to the RNA polymerase beta' chain family.

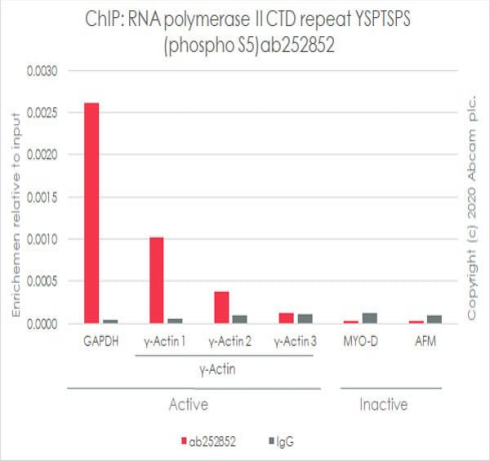
ドメイン The C-terminal domain (CTD) serves as a platform for assembly of factors that regulate transcription initiation, elongation, termination and mRNA processing.

翻訳後修飾 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.

細胞内局在 Nucleus.

画像

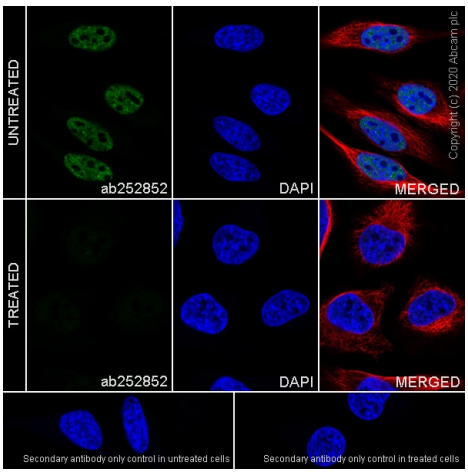


ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] (ab252852)

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 ab252852 (red), or 5 µg of Rat IgG2a **ab18450** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

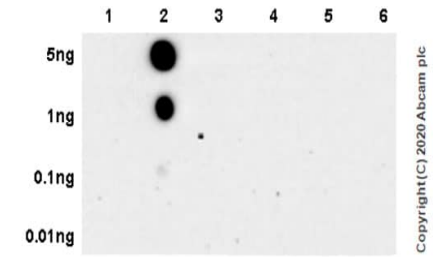
***https://www.abcam.com/resources?keywords=X%20ChIP%20protocol**



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] - ChIP Grade (ab252852)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling RNA polymerase II CTD repeat YSPTSPS (phospho S5) with ab252852 at 1/500 (1.29 µg/mL) dilution , followed by **ab150157** Goat Anti-Rat IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 dilution (Green). Confocal image showing nuclear staining in HeLa cell line, the signal decreased after phosphatase treatment at 37°C for 2h. **ab179513** Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/500 dilution, followed by **ab150080** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) at 1/500 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150157** Goat Anti-Rat IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] (ab252852)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) labeled with ab252852 at 1/1000 (0.645 µg/mL) dilution.

Goat Anti-Rat IgG (H+L), HRP) (**ab205720**) at 1/5000 dilution was used as secondary antibody.

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

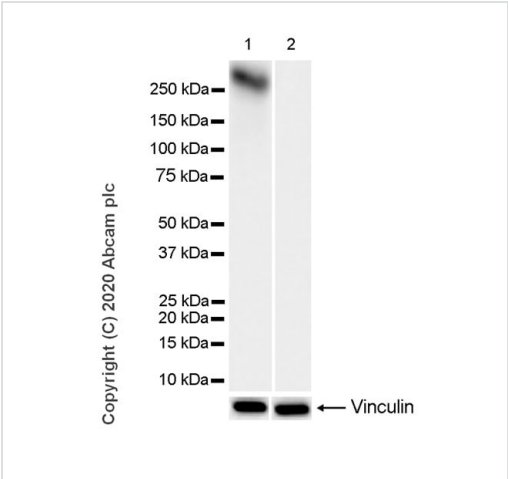
Lane 4: RNA polymerase II CTD repeat YSPTSPS (phospho Y1) peptide

Lane 5: RNA polymerase II CTD repeat YSPTSPS (phospho S7) peptide

Lane 6: RNA polymerase II CTD repeat YSPTSPS non-phopho peptide

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] (ab252852)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] - ChIP Grade (ab252852) at 0.645 µg/ml

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.

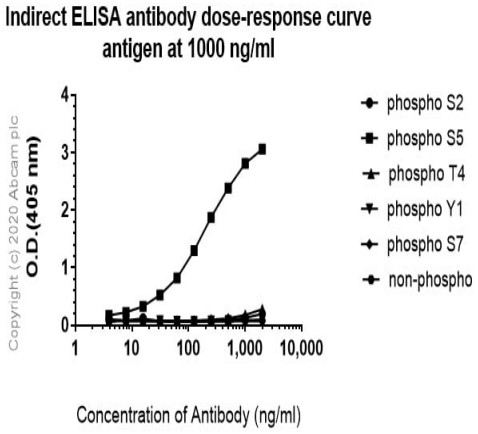
Secondary
All lanes : Goat Anti-Rat IgG (H+L), HRP) (**ab205720**) at 1/5000 dilution

Predicted band size: 217 kDa
Observed band size: 260 kDa

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.

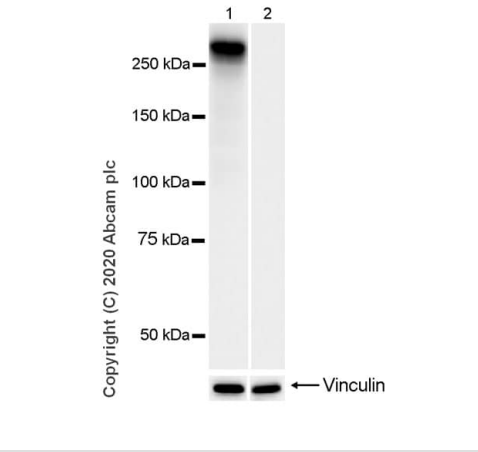


Indirect ELISA - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] - ChIP Grade (ab252852)

ab252852 was used at 0 - 1000 ng/mL.

Antigens were used at 1000 ng/mL.

An Alkaline Phosphatase-conjugated Anti-Rat IgG (H+L) wasused as secondary antibody at 1/1000 dilution.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] (ab252852)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] - ChIP Grade (ab252852) at 0.645 µg/ml

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

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

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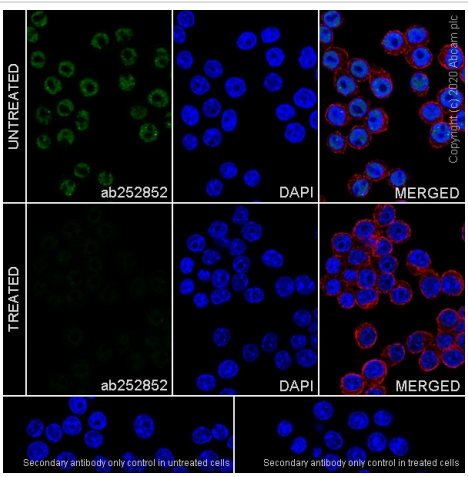
Exposure time: 3 seconds.



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Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW264.7 cells labelling RNA polymerase II CTD repeat YSPTSPS (phospho S5) with ab252852 at 1/500 (1.29 µg/mL) dilution, followed by **ab150157** Goat Anti-Rat IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in RAW264.7 cell line, the signal decreased after phosphatase treatment at 37°C for 2h. **ab179513** Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/500 dilution, followed by **ab150080** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) secondary antibody at 1/500 dilution (Red). The Nuclear counterstain was DAPI (Blue).

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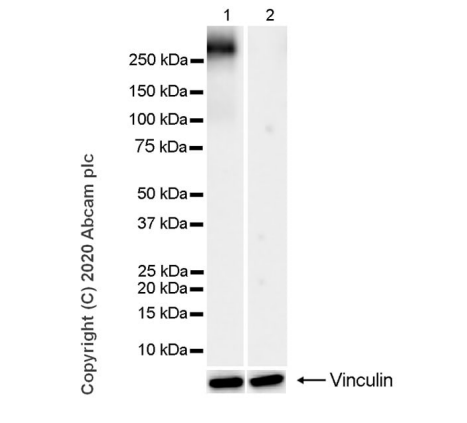


Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] (ab252852)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [3E8] - ChIP Grade (ab252852) at 0.645 µg/ml

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.

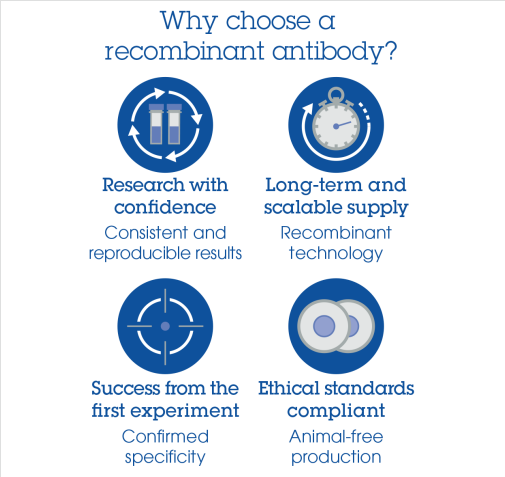
Secondary
All lanes : Goat Anti-Rat IgG (H+L), HRP) (**ab205720**) at 1/5000 dilution

Predicted band size: 217 kDa
Observed band size: 260 kDa

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: 37 seconds.



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