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Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade ab5408

23 Abreviews 342 References 画像数 9

製品の概要

製品名	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade
製品の詳細	Mouse monoclonal [4H8] to RNA polymerase II CTD repeat YSPTSPS (phospho S5) - ChIP Grade
由来種	Mouse
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, Dot blot, ChIP, WB, ELISA
種交差性	**交差種:** Mouse, Rat, Human **交差が予測される動物種:** Arabidopsis thaliana
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	Flow Cyt: HeLa cells. WB: MCF7, HEK-293T and NIH/3T3 whole cell lysate. ICC/IF: HeLa and MCF7 cells. Dot Blot: RNA polymerase II CTD repeat YSPTSPS (phospho S5) phospho peptide. ChIP: U-2 OS cells.
特記事項	**ChIP protocols:** **ChIP protocol for cross-linking ChIP (X-ChIP)** **Native ChIP protocol** **Chromatin preparation from tissues for ChIP** **ChIP troubleshooting** **ChIP tips and tricks guide** This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. 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, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.50 Preservative: 0.02% Sodium azide Constituent: PBS Contains 0.4M Arginine
精製度	Protein G purified
ポリ/モノ	モノクローナル
クローン名	4H8
ミエローマ	Sp2/0-Ag14
アイソタイプ	IgG1

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ab5408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
Flow Cyt (Intra)		Use 1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (9)	1/1000.
Dot blot		1/1000.
ChIP	★★★★☆ (7)	Use 1-4µg for 10^6 cells.
WB	★★★★☆ (4)	1/1000. Detects a band of approximately 260 kDa (predicted molecular weight: 217 kDa).
ELISA		Use at an assay dependent concentration.

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.

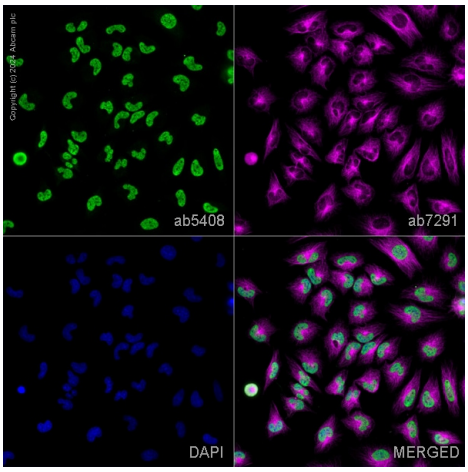
配列類似性 Belongs to the RNA polymerase beta' chain family.

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.

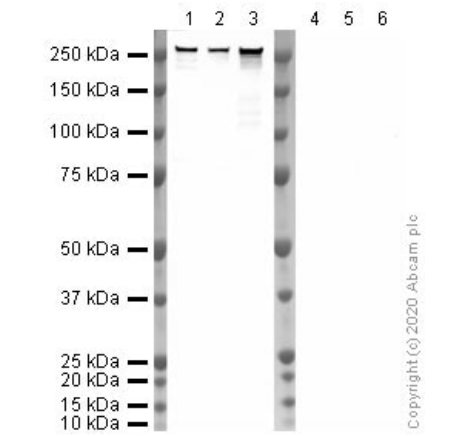
細胞内局在 Nucleus.

画像



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

ab5408 staining RNA polymerase II CTD repeat YSPTSPS (phospho S5) in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab5408 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 4% paraformaldehyde (10 min).Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/1000 dilution

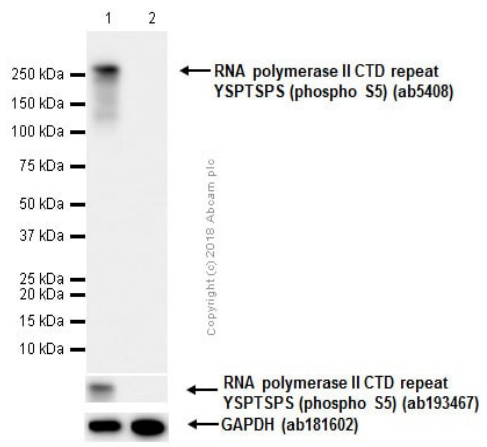
Lane 1 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate (**ab28419**)
Lane 2 : NIH 3T3 (Mouse) Whole Cell Lysate (**ab52956**)
Lane 3 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate (**ab50957**)
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate (**ab28419**) Then the membrane was incubated with alkaline phosphatase
Lane 5 : NIH 3T3 (Mouse) Whole Cell Lysate (**ab52956**) Then the membrane was incubated with alkaline phosphatase
Lane 6 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate (**ab50957**) Then the membrane was incubated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-mouse IgG at 1/10000 dilution

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

Exposure time: 8 minutes



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/1000 dilution (purified)

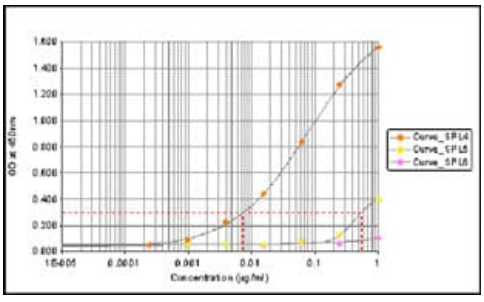
Lane 1 : MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate. Then the membrane was incubated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-mouse IgG at 1/10000 dilution

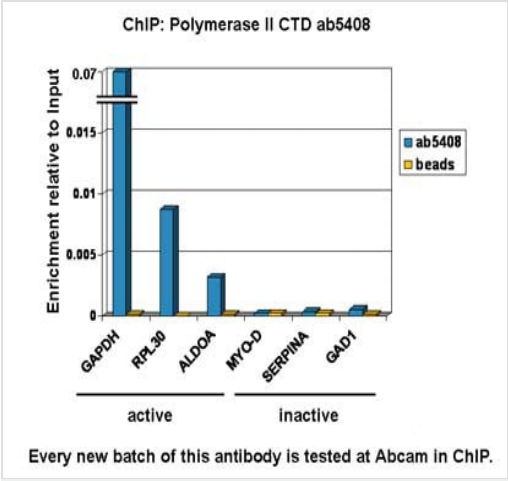
Predicted band size: 217 kDa
Observed band size: 270 kDa

Blocking/Diluting Buffer and concentration



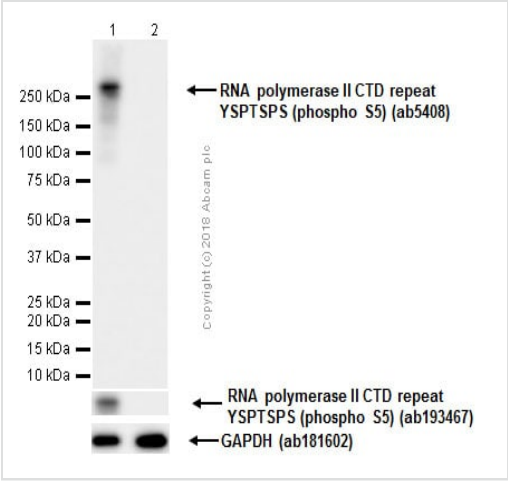
ELISA - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

ELISA using ab5408 at varying antibody concentrations. Curve_SPL4 indicates binding to RNA polymerase II CTD repeat YSPTSPS peptide - phospho S5 (**ab18488**). Binding to the following peptides was much weaker: Curve_SPL5 RNA polymerase II CTD repeat YSPTSPS peptide - phospho S2 (**ab12793**), Curve_SPL6 RNA polymerase II CTD repeat YSPTSPS peptide (**ab12795**).



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

Chromatin was prepared from U-2 OS (human bone osteosarcoma epithelial cell line) cells according to the **Abcam X-ChIP protocol**. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 2 µg of ab5408 (blue), and 20 µl of protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/1000 dilution

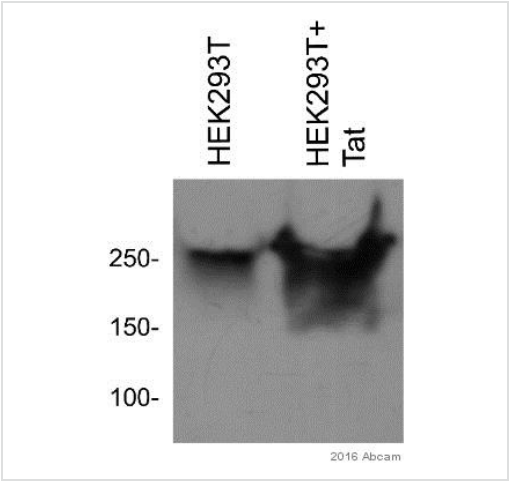
Lane 1 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate
Lane 2 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate. Then the membrane was incubated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-mouse IgG at 1/10000 dilution

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

Blocking/Diluting Buffer and concentration 5% NFDM/TBST



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

This image is courtesy of an anonymous Abreview.

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/800 dilution

Lane 1 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 2 : HEK293T transfected with Tat containing plasmid whole cell lysate

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-mouse IgG at 1/5000 dilution

Performed under reducing conditions.

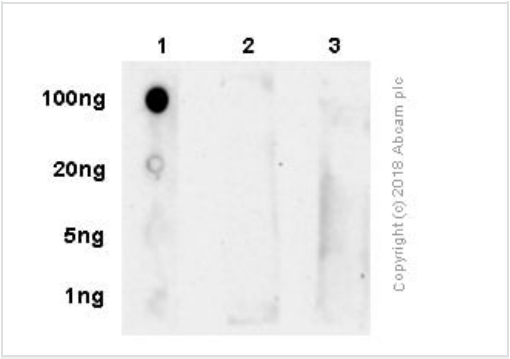
Predicted band size: 217 kDa
Observed band size: 260 kDa

Exposure time: 30 seconds

7.5% SDS gel.

Blocked with 5% BSA for 1 hour at 25°C.

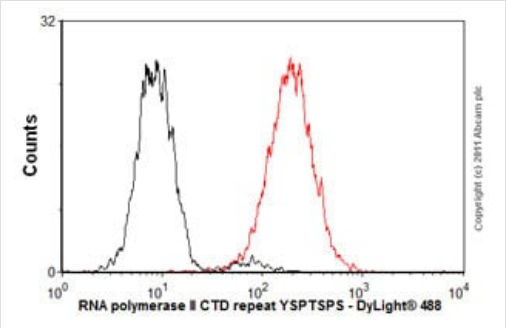
Incubated with the primary antibody for 3 hours at 25°C in 1X TBST buffer.



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

Dot blot analysis of RNA polymerase II CTD repeat YSPTSPS (phospho S5) phospho peptide (Lane 1), RNA polymerase II CTD repeat YSPTSPS (phospho 2) phospho peptide (Lane 2) and RNA polymerase II CTD repeat YSPTSPS non-phospho peptide (Lane 3) labeling RNA polymerase II CTD repeat YSPTSPS (phospho S5) phospho peptide with ab5408 at a dilution of 1/1000 dilution (1ug/ml). A HRP-conjugated goat anti-mouse IgG was used as the secondary antibody at a dilution of 1/10,000 dilution.

Blocking buffer: 5% NFDM/TBST. Dilution buffer: 5% NFDM /TBST .



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab5408 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab5408, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was goat **anti-mouse DyLight® 488** (IgG H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

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