abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] ab252854

リコンビナント

2 References 画像数 8

製品の概要

製品名	Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7]
製品の詳細	Rat monoclonal [1C7] to RNA polymerase II CTD repeat YSPTSPS
由来種	Rat
アプリケーション	**適用あり:** WB, ChIP, IP, Dot blot, Indirect ELISA **適用なし:** ICC
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: Raji and RAW264.7, PC-12 lysates. IP: and Raji, RAW264.7 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	1C7

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ab252854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
WB		Use a concentration of 0.584 µg/ml. Predicted molecular weight: 217 kDa.
ChIP		Use 5 µg for 25 µg of chromatin.
IP		Use a concentration of 19.467 µg/ml.
Dot blot		Use a concentration of 0.584 µg/ml.
Indirect ELISA		Use a concentration of 9.419 µg/ml.

追加情報 Is unsuitable for ICC.

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.

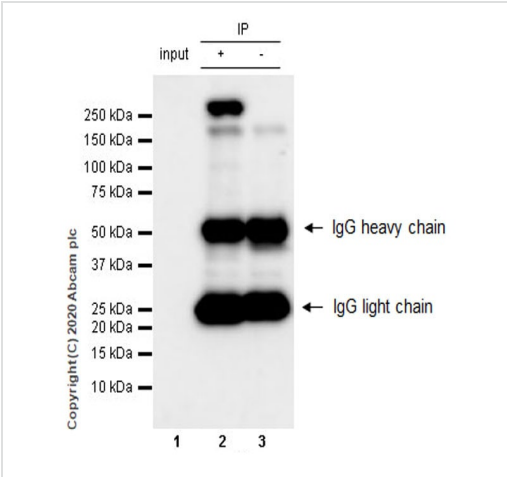
翻訳後修飾 The tandem heptapeptide repeats in the C-terminal domain (CTD) can be highly phosphorylated. The phosphorylation activates Pol II. Phosphorylation occurs mainly at residues 'Ser-2' and 'Ser-5' of the heptapeptide repeat and is mediated, at least, by CDK7 and CDK9. CDK7 phosphorylation of POLR2A 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



Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854)

RNA polymerase II CTD repeat YSPTSPS was immunoprecipitated from 0.35 mg Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate with ab252854 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab252854 at 1/1000 dilution. Goat Anti-Rat IgG (H+L), HRP) (**ab205720**) was used at 1/5000 dilution.

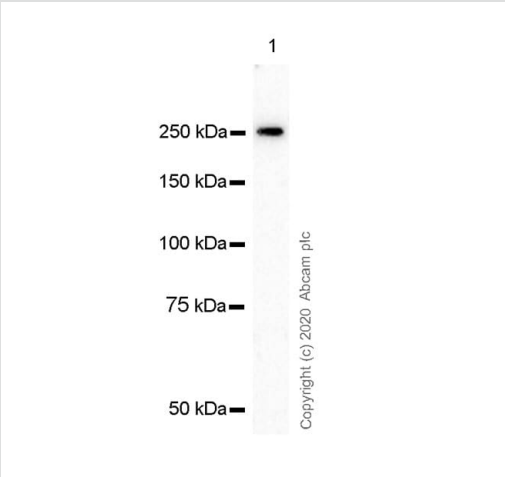
Lane 1: Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate 10 ug

Lane 2: ab252854 IP in Raji whole cell lysate

Lane 3: Rat monoclonal IgG2a (**ab18450**) instead of ab252854 in Raji whole cell lysate

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

Exposure time: 24 seconds



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854)

Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854) at 1/1000 dilution + PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate at 10 µg

Secondary

Goat Anti-Rat IgG H&L (HRP) (**ab205720**) at 1/5000 dilution

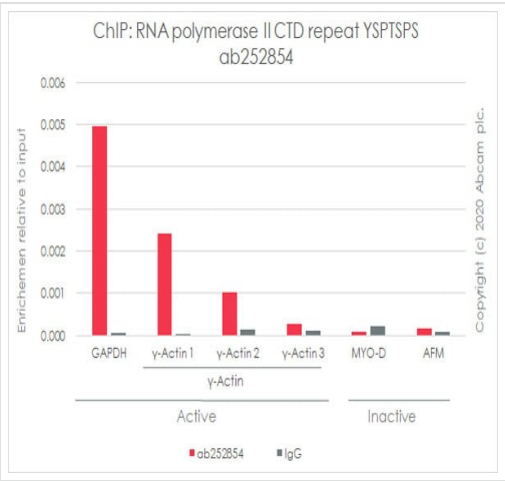
Predicted band size: 217 kDa

Observed band size: 250 kDa

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

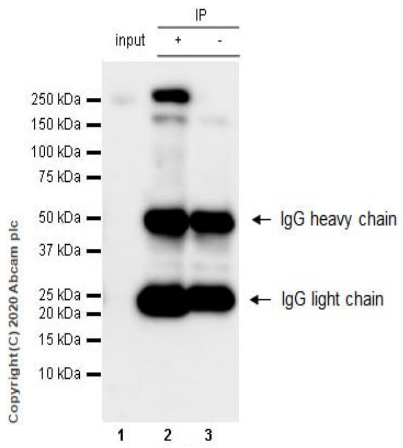
The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 22745433).

Exposure time: 3 minutes



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854)

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 ab252854 (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).
http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854)

RNA polymerase II CTD repeat YSPTSPS was immunoprecipitated from 0.35 mg RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate with ab252854 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab252854 at 1/1000 dilution. Goat Anti-Rat IgG (H+L), HRP) (**ab205720**) was used at 1/5000 dilution.

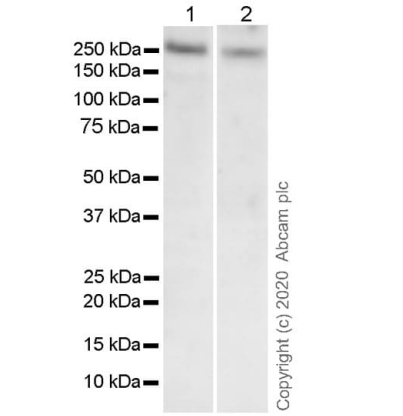
Lane 1: RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate 10 ug

Lane 2: ab252854 IP in RAW264.7 whole cell lysate

Lane 3: Rat monoclonal IgG2a (**ab18450**) instead of ab252854 in RAW264.7 whole cell lysate

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

Exposure time: 24 seconds



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854) at 1/1000 dilution

Lane 1 : Raji (human Burkitts lymphoma B lymphocyte), whole cell lysate
Lane 2 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate

Lysates/proteins at 20 µg per lane.

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

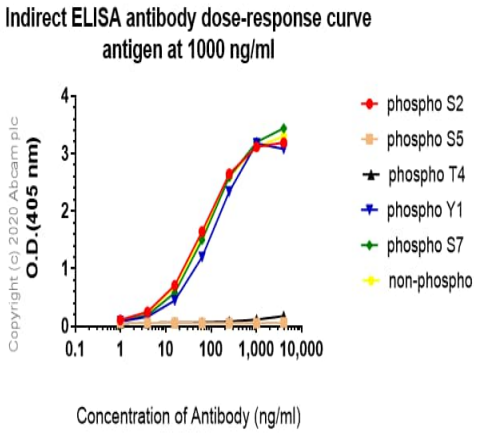
Predicted band size: 217 kDa
Observed band size: 250 kDa

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)

This blot was developed using a higher sensitivity ECL substrate.

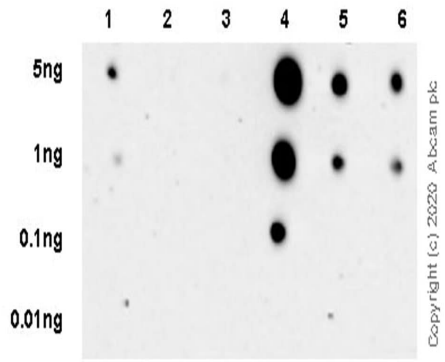
Exposure time: 37 seconds



Indirect ELISA - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854)

ELISA analysis using ab252854 at a range of 4000-0 ng/ml followed by a Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rat IgG (H+L) at 1/1000 dilution.

Antigens: phospho S2, phospho S5, phospho T4, phospho Y1, phospho S7, non-phospho



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854)

Dot blot analysis of ab252854 at 1/1000 dilution followed by a Goat Anti-Rat IgG (H+L), HRP) (**ab205720**) Secondary antibody at 1/5000 diluton. The observed data is consistent with what has been described in the literature (PMID: 22549466). Exposure trime: 3 minutes.

Blocking and diluting buffer: 5% NFDM/TBST

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

Anti-RNA polymerase II CTD repeat YSPTSPS antibody [1C7] (ab252854)

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