abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) ab300575

Recombinant RabMAb

1 References 12 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade)
Description	Rabbit monoclonal [EPR24494-59] to RNA polymerase II CTD repeat YSPTSPS - ChIP Grade
Host species	Rabbit
Specificity	This antibody is unsuitable for mouse IP.
Tested applications	**Suitable for:** WB, IP, ChIP, Flow Cyt (Intra), Dot blot, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Whole cell lysates: HeLa (human cervix adenocarcinoma epithelial cell), 293T (human embryonic kidney epithelial cell), NIH/3T3 (mouse embryonic fibroblast), PC-12 (rat adrenal gland pheochromocytoma), RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage). IHC-P: Human, mouse, and rat colon. ICC/IF: HeLa, RAW 264.7. Flow cyt. Intra.: HeLa, RAW 264.7. IP: HeLa. ChIP: Hela. DB: RNA polymerase II CTD repeat YSPTSPS non-phosphorylated and (phospho Y1/S7) peptide.
General notes	ab300575 does not react in immunoprecipitation with mouse. 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: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR24494-59
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab300575 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

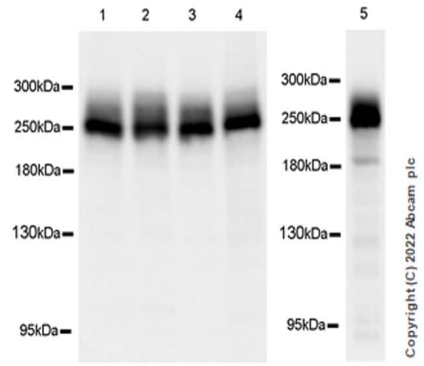
Application	Abreviews	Notes
WB		1/5000.
IP		1/30.
ChIP		Use 5 µg for µg of chromatin.
Flow Cyt (Intra)		1/500.
Dot blot		1/1000.
IHC-P		1/5000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/50.

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 antibody [EPR24494-59] (ChIP Grade) (AB300575)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (ab300575) at 1/5000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : 293T (human embryonic kidney epithelial cell) whole cell lysate
Lane 3 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 4 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate
Lane 5 : RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate

Lysates/proteins at 20 µg per lane.

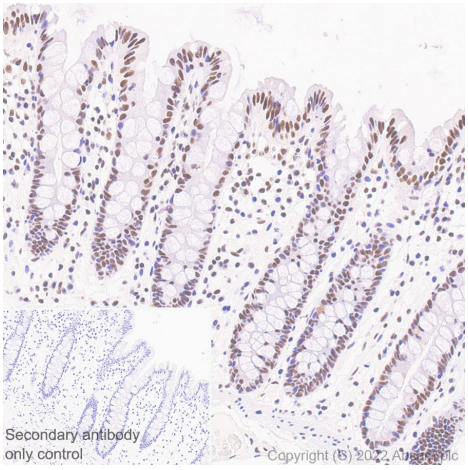
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Observed band size: 250 kDa

Exposure time: 3 seconds

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

Exposure time: 3 seconds

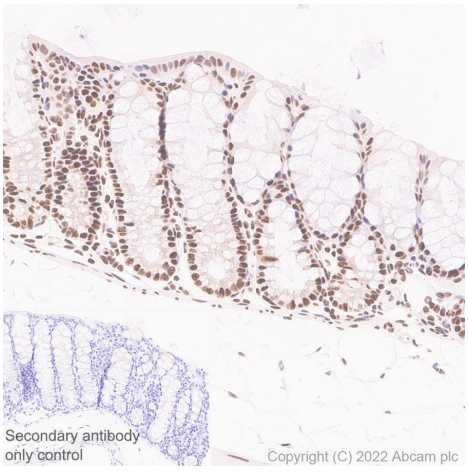


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (AB300575)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab300575at 1/5000 dilution (0.108 µg/mL) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection kit). Nuclear staining on human colon is observed (PMID: 25901683). The section was incubated with ab300575 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody, followed by a ready to use secondary antibody LeicaDS9800 (Bond™ Polymer Refine Detection kit).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (AB300575)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/5000 dilution (0.108 µg/mL) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection kit). Nuclear staining on mouse colon is observed. The section was incubated with ab300575 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody, followed by a ready to use secondary antibody LeicaDS9800 (Bond™ Polymer Refine Detection kit).

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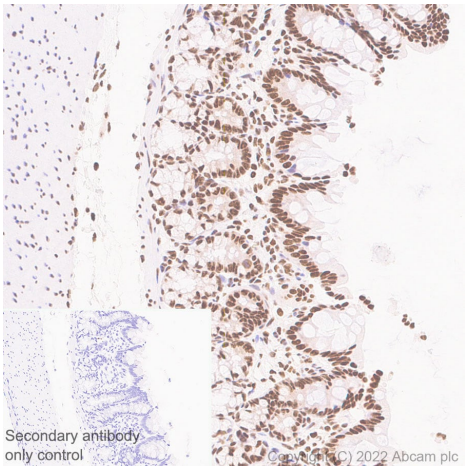
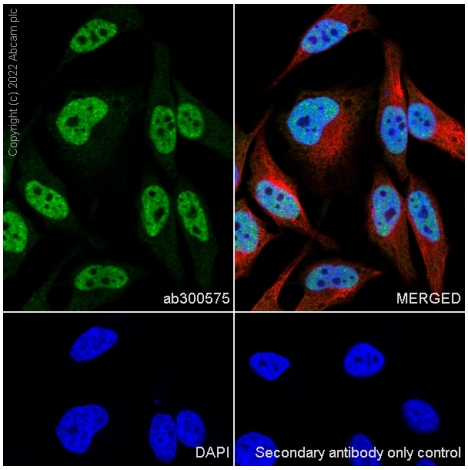


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (AB300575)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/5000 dilution (0.108 µg/mL) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection kit). Nuclear staining on rat colon. The section was incubated with ab300575 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody, followed by a ready to use secondary antibody LeicaDS9800 (Bond Polymer Refine Detection kit).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.

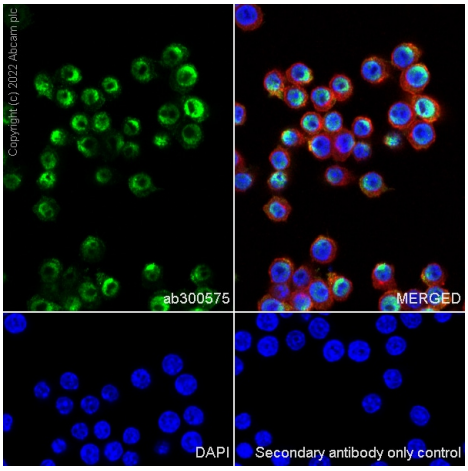


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (ab300575)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cells) labeling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/50 dilution (10.78 µg/ml), followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing mainly nuclear staining in HeLa cell line.

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 µg/ml) (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: PBS was used instead of primary antibody, followed by a preadsorbed secondary antibody **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (2 µg/mL).



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (ab300575)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) cells labeling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/50 (10.78 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing mainly nuclear staining in RAW 264.7 cell line.

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 µg/ml) (Red). The Nuclear counterstain was DAPI (Blue).

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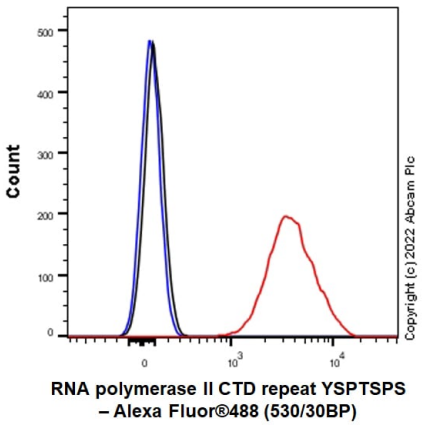
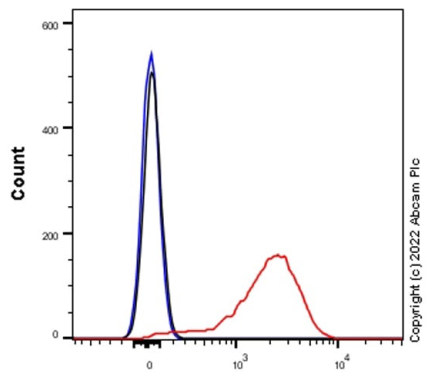


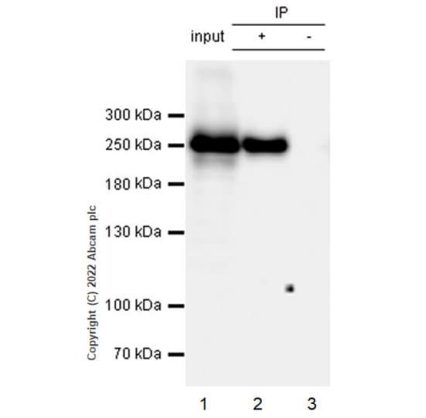
Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (AB300575)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cells) labelling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/500 dilution (0.1 µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (AB300575)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/500 dilution (0.1 µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (AB300575)

RNA polymerase II CTD repeat YSPTSPS was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) 2 µg whole cell lysate with ab300575 at 1/30 dilution (2 µg in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using ab300575 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

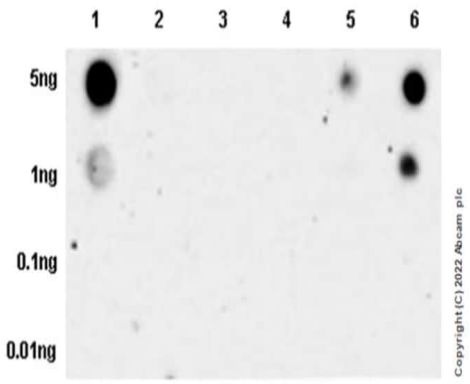
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 2 µg (Input)

Lane 2: ab300575 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab300575 in HeLa whole cell lysate

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

Exposure time: 10 seconds



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (ab300575)

Dot blot analysis of RNA polymerase II CTD repeat YSPTSPS using ab300575 at 1:1000 (0.539 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1:100,000 dilution.

Lane 1: RNA polymerase II CTD repeat YSPTSPS (phospho Y1) peptide

Lane 2: RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide

Lane 3: RNA polymerase II CTD repeat YSPTSPS (phospho T4) peptide

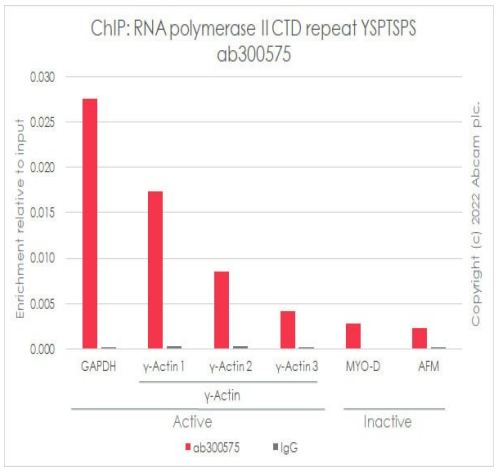
Lane 4: RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide

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

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

Exposure time: 3 minutes

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



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (AB300575)

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 10 min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab300575 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers and probes are located in the first kb of the transcribed region
*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol

Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (AB300575)

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