abcam

ab300576

Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free

Recombinant RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR24494-59
Purification technique	Affinity purification Protein A
Specificity	This antibody is unsuitable for mouse IP.
Concentration	1.002 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Rat, Mouse, Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Expected

Species	Rat, Mouse, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.

Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Rat, Mouse, Human

Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Target data

See full target information POLR2A

Function

Catalytic core component of RNA polymerase II (Pol II), a DNA-dependent RNA polymerase which synthesizes mRNA precursors and many functional non-coding RNAs using the four ribonucleoside triphosphates as substrates (By similarity) (PubMed:23748380, PubMed:27193682, PubMed:30190596, PubMed:9852112). Pol II-mediated transcription cycle proceeds through transcription initiation, transcription elongation and transcription termination stages. During transcription initiation, Pol II pre-initiation complex (PIC) is recruited to DNA promoters, with focused-type promoters containing either the initiator (Inr) element, or the TATA-box found in cell-type specific genes and dispersed-type promoters that often contain hypomethylated CpG islands usually found in housekeeping genes. Once the polymerase has escaped from the promoter it enters the elongation phase during which RNA is actively polymerized, based on complementarity with the template DNA strand. Transcription termination involves the release of the RNA transcript and polymerase from the DNA (By similarity) (PubMed:23748380, PubMed:27193682, PubMed:28108474, PubMed:30190596, PubMed:9852112). Forms Pol II active center together with the second largest subunit POLR2B/RPB2. Appends one nucleotide at a time to the 3' end of the nascent RNA, with POLR2A/RPB1 most likely contributing a Mg(2+)-coordinating DxDGD motif, and POLR2B/RPB2 participating in the coordination of a second Mg(2+) ion and providing lysine residues believed to facilitate Watson-Crick base pairing between the incoming nucleotide and template base. Typically, Mg(2+) ions direct a 5' nucleoside triphosphate to form a phosphodiester bond with the 3' hydroxyl of the preceding nucleotide of the nascent RNA, with the elimination of pyrophosphate. The reversible pyrophosphorolysis can occur at high pyrophosphate concentrations (By similarity) (PubMed:30190596, PubMed:8381534, PubMed:9852112). Can proofread the nascent RNA transcript by means of a 3' -> 5' exonuclease activity. If a ribonucleotide is mis-incorporated, backtracks along the template DNA and cleaves the phosphodiester bond releasing the mis-incorporated 5'-ribonucleotide (By similarity) (PubMed:8381534). Through its unique C-terminal domain (CTD, 52 heptapeptide tandem repeats) serves as a platform for assembly of factors that regulate transcription initiation, elongation and termination. CTD phosphorylation on Ser-5 mediates Pol II promoter escape, whereas phosphorylation on Ser-2 is required for Pol II pause release during transcription elongation and further pre-mRNA processing. Additionally, the regulation of gene expression levels depends on the balance between methylation

and acetylation levels of the CTD-lysines. Initiation or early elongation steps of transcription of growth-factor-induced immediate early genes are regulated by the acetylation status of the CTD. Methylation and dimethylation have a repressive effect on target genes expression. Cooperates with mRNA splicing machinery in co-transcriptional 5'-end capping and co-transcriptional splicing of pre-mRNA (By similarity) (PubMed:24207025, PubMed:26124092). RNA-dependent RNA polymerase that catalyzes the extension of a non-coding RNA (ncRNA) at the 3'-end using the four ribonucleoside triphosphates as substrates. An internal ncRNA sequence near the 3'-end serves as a template in a single-round Pol II-mediated RNA polymerization reaction. May decrease the stability of ncRNAs that repress Pol II-mediated gene transcription. (Microbial infection) Acts as an RNA-dependent RNA polymerase when associated with small delta antigen of Hepatitis delta virus, acting both as a replicase and transcriptase for the viral RNA circular genome.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

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.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	RNA polymerase II CTD repeat YSPTSPS also known as the C-terminal domain of RNA polymerase II is a critical component of the RNA polymerase II enzyme commonly referred to as pol II. This domain is characterized by the repetitive sequence YSPTSPS which plays a significant role in the regulation of transcription. The mass of RNA polymerase II including its CTD varies but is essential for its function in gene expression. RNA polymerase II with the CTD is expressed in the nucleus of eukaryotic cells where it orchestrates the transcription of DNA into mRNA.
Biological function summary	RNA polymerase II CTD repeat YSPTSPS is essential for the transcription progression from initiation to termination. It is part of the large RNA polymerase II complex interacting with various transcription factors and enzymes necessary for RNA processing. The phosphorylation state of the CTD particularly on serine residues regulates interactions with splicing machinery and other RNA processing factors. This modulation ensures the coupling between transcription and RNA processing events controlling mRNA synthesis and maturation.
Pathways	RNA polymerase II CTD repeat YSPTSPS is important in the mRNA synthesis pathway specifically in transcriptional regulation and processing of nascent RNA transcripts. It interacts with proteins such as the transcription factors TFIIH and TFIIB which aid in promoter recognition and open complex formation. The CTD's dynamic phosphorylation pattern allows integration into multiple cellular pathways most importantly connecting transcription with RNA splicing and transport pathways.
Associated diseases and disorders	Abnormal function or mutations in RNA polymerase II CTD repeat YSPTSPS associate with diseases such as transcription-related syndromes and certain cancers. Deficient CTD phosphorylation can lead to improper mRNA processing resulting in neural developmental disorders. Additionally its interaction with proteins like CDK7 which phosphorylates the CTD links it to tumors where transcriptional dysregulation is a hallmark. Understanding the CTD's role in these diseases provides insight into therapeutic targets and strategies for intervention.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

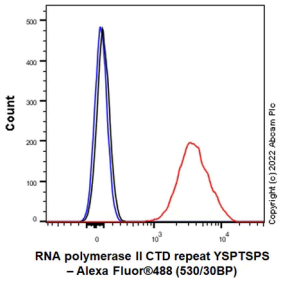
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

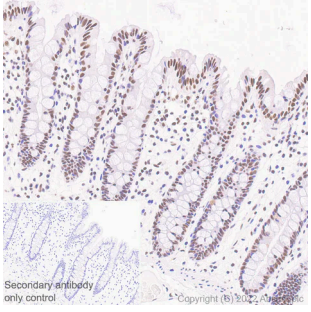
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11 product images



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

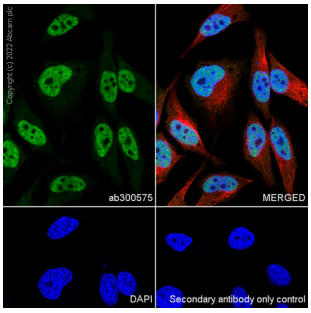
This data was developed using ab300575, the same antibody clone in a different buffer formulation. Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/500 dilution (0.1ug) (Red) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

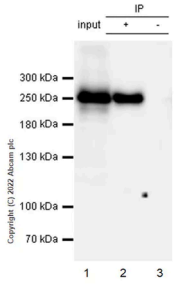
This data was developed using ab300575, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/5000 (0.108 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Nuclear staining on human colon (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: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

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Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

This data was developed using ab300575, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling 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 2ug/mL dilution (Green). Confocal image showing mainly nuclear staining in HeLa cell line is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/mL dilution.



Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

This data was developed using ab300575, the same antibody clone in a different buffer formulation. RNA polymerase II CTD repeat YSPTSPS was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 2 ug with ab300575 at 1/30 dilution (2ug in 0.35mg 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 ug

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

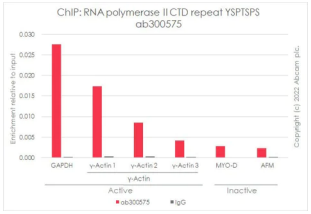
All lanes:
Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] (ChIP Grade) (ab300575) at 1/1000 dilution

All lanes:
HeLa whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

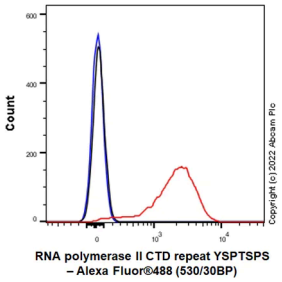
Exposure time: 10s



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

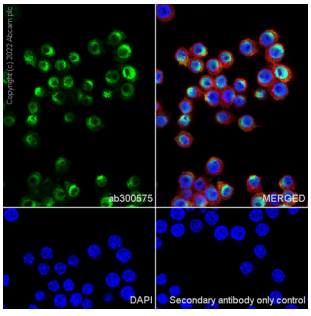
This data was developed using ab300575, the same antibody clone in a different buffer formulation. Chromatin was prepared from HeLa cells according to the Abcam Dual-X-ChIP protocol. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 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.



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

This data was developed using ab300575, the same antibody clone in a different buffer formulation. 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 (0.1ug) (Red) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled 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.



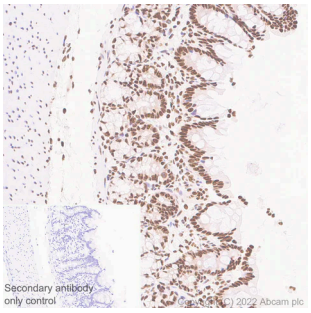
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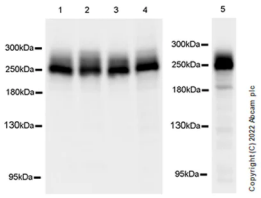
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

This data was developed using ab300575, the same antibody clone in a different buffer formulation. Immunohistochemical analysis of paraffin-embedded Rat colon tissue labeling RNA polymerase II CTD repeat YSPTSPS with ab300575 at 1/5000 (0.108 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. 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: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

This data was developed using ab300575, the same antibody clone in a different buffer formulation. Blocking Buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - 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

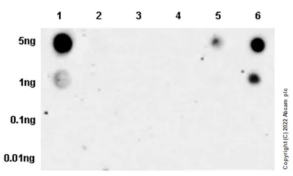
Lane 5:
RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate

Secondary

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

Observed band size: 250 kDa

Exposure time: 3s



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS antibody [EPR24494-59] - ChIP Grade - BSA and Azide free (ab300576)

This data was developed using ab300575, the same antibody clone in a different buffer formulation.

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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.