abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade ab5095

4.4 44 Abreviews 323 References 13 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade
Description	Rabbit polyclonal to RNA polymerase II CTD repeat YSPTSPS (phospho S2) - ChIP Grade
Host species	Rabbit
Specificity	This antibody recognises the phosphorylated serine found in the amino acid 2 position of the C-terminal domain repeat YSPTSPS.
Tested applications	**Suitable for:** ELISA, IHC-FoFr, ChIP, ChIP/Chip, IHC-P, IHC-Fr, ICC/IF, IHC - Wholemount, Dot blot, WB, CHIPseq
Species reactivity	**Reacts with:** Mouse, Rat, Human, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe **Predicted to work with:** a wide range of other species
Immunogen	**This product was produced with the following immunogens:** Synthetic peptide corresponding to Saccharomyces cerevisiae RNA polymerase II CTD repeat YSPTSPS aa 1600-1700 conjugated to keyhole limpet haemocyanin. Database link: P04050 (Peptide available as ab12793, ab57366) Synthetic peptide corresponding to Human RNA polymerase II CTD repeat YSPTSPS conjugated to keyhole limpet haemocyanin. (Peptide available as ab12793, ab57366) Synthetic peptide corresponding to Human RNA polymerase II CTD repeat YSPTSPS conjugated to keyhole limpet haemocyanin. (Peptide available as ab12793, ab57366)
Positive control	WB: Hela Whole Cell Lysate and S.cerevisiae extract. IHC-P: Human pancreas, brain and tonsil tissue, mouse and rat kidney tissue. ICC-IF: MCF7 and HeLa cells. IHC-FoFr: Rat Brain tissue ChIP: Mouse RAW macrophages nuclear cell lysate
General notes	

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab5095** 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
ELISA	★★★★☆	1/438.
IHC-FoFr	★★★★★	Use at an assay dependent concentration.
ChIP	★★★★☆	Use at an assay dependent concentration.
ChIP/Chip		Use at an assay dependent concentration.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr	★★★★★	Use at an assay dependent concentration.
ICC/IF	★★★★☆	Use a concentration of 1 - 5 µg/ml.
IHC - Wholemount	★★★★★	Use at an assay dependent concentration.
Dot blot		1/3000. AbReview 31067
WB	★★★★☆	Use a concentration of 1 µg/ml. Detects a band of approximately 240 kDa (predicted molecular weight: 217 kDa).Can be blocked with *S. cerevisiae* RNA polymerase II CTD repeat YSPTSPS (phospho S1606 + S1613) peptide (ab12793).
CHIPseq		Use 2-0.3 µg for µg of chromatin.

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.

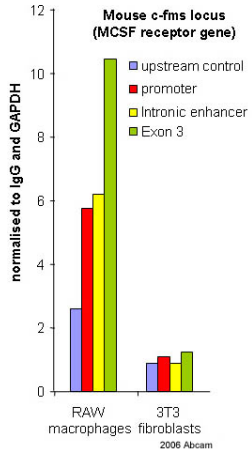
Post-translational modifications

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.

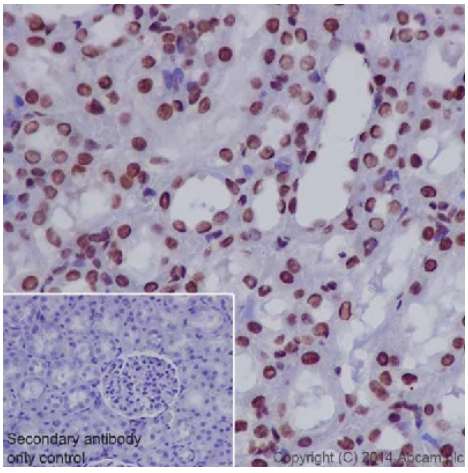
Images



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

This image is courtesy of an anonymous Abreview

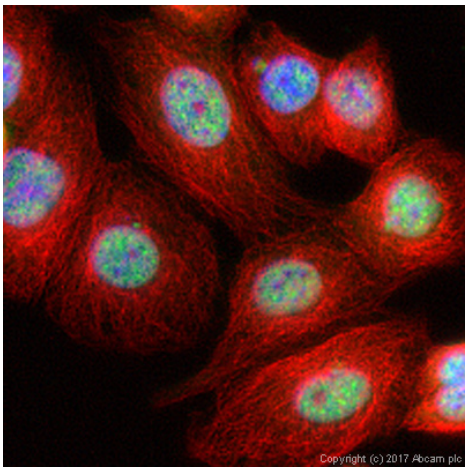
ab5095 at 4ug/ml in ChIP of RAW macrophages. Nuclear cell lysate of mouse RAW macrophages (expressing c-fms) were formaldehyde cross linked and ChIP tested with ab5095. The nuclear preparation was frozen before sonication with a probe sonicator. All buffers used contained protease inhibitors. 3T3 fibroblasts (not expressing c-fms) were used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

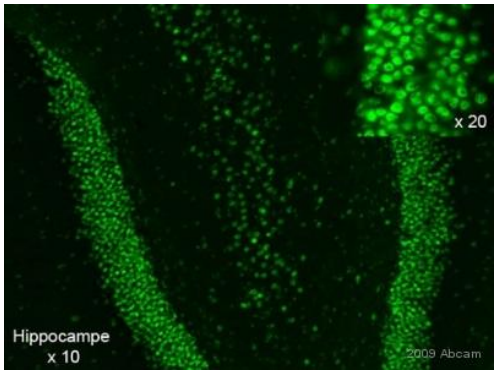
Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab5095 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Weak nuclear staining on epithelium cells and glomerulus cells of rat kidney was observed. Counter stained with Hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH9.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

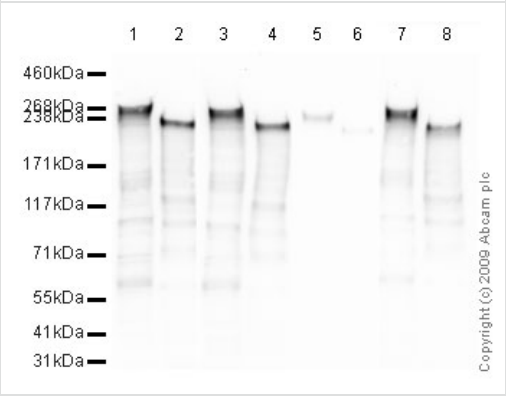
ab5095 stained in MCF7 cells. Cells were fixed with 100% methanol (5 min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab5095 at 1µg/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150120 (pseudo-colored red) and ab150081 (colored green) used at 1 µg/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1hour at room temperature.



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

This image is courtesy of an Abreview submitted by Karine Thibault.

Ab5095 staining RNA polymerase II CTD repeat YSPTSPS (phospho S2) in Rat Brain tissue sections by IHC-FoFr (PFA perfusion fixed frozen sections). Tissue was fixed with paraformaldehyde and samples were incubated with primary antibody (1/3000 in 0.3% PBS-T) for 18hours at 20°C. An Alexa Fluor® 488 Anti-IgG Mouse polyclonal was used as the secondary antibody at 1/1000 dilution.



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095) at 1 µg/ml

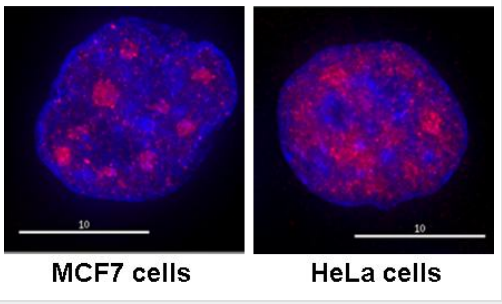
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : S.cerevisiae (Y190) Whole Cell Lysate
Lane 3 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS peptide (ab12795) at 1 µg/ml
Lane 4 : S.cerevisiae (Y190) Whole Cell Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS peptide (ab12795) at 1 µg/ml
Lane 5 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS (phospho S1606 + S1613) peptide (ab12793) at 1 µg/ml
Lane 6 : S.cerevisiae (Y190) Whole Cell Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS (phospho S1606 + S1613) peptide (ab12793) at 1 µg/ml
Lane 7 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide (ab18488) at 1 µg/ml
Lane 8 : S.cerevisiae (Y190) Whole Cell Lysate with Human RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide (ab18488) at 1 µg/ml

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

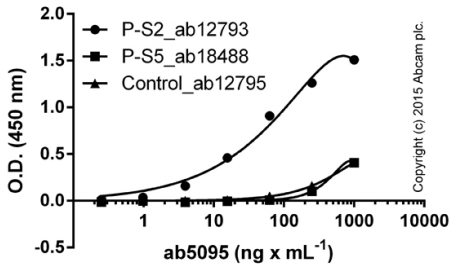
Predicted band size: 217 kDa
Observed band size: 240 kDa
why is the actual band size different from the predicted?



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

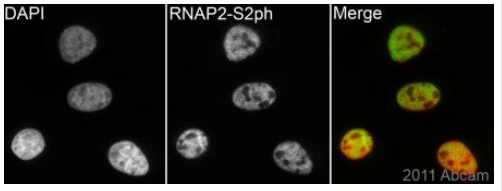
This image is courtesy of Michael Mancini, Baylor College of Medicine

HeLa or MCF7 cells were fixed with 4% formaldehyde in PEM buffer. The coverslip was incubated in blocking buffer of 5% powdered milk in TBS-T plus 0.02% sodium azide for 1 hour at room temperature. Blocking buffer was removed and primary antibody was added at a dilution of 1/500 and incubated overnight at 4 degrees celsius. The coverslips were then washed 4-5 times with blocking buffer for 5 minutes. Secondary antibody, goat anti-rabbit Alexa 594, was added at a dilution of 1/1000 and incubated at room temperature for one hour. From this point on coverslips were covered with foil to protect them from light. They were washed 5 times with TBS-T and then one time with PEM, for 5 minutes each wash. The coverslips were fixed 10-30 minutes in 4% formaldehyde in PEM buffer, then washed 3 times with PEM buffer for 5 minutes. 0.1M ammonium chloride in PEM buffer was added for 10 minutes to quench auto-florescence, and then slips were washed 2 times for 5 minutes in PEM followed by 3 washes for



ELISA - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

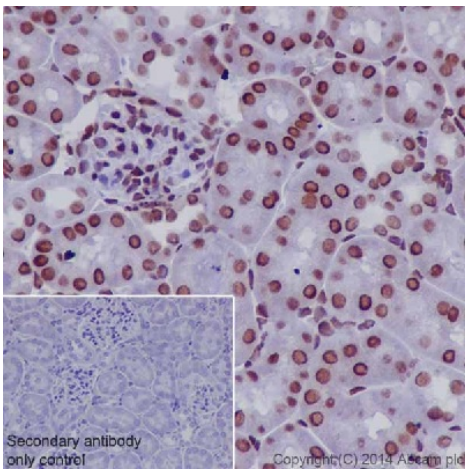
Diluted ab5095 was bound to immobilised phospho- or control peptides (1 microgram per mL). The antibody was detected by goat anti-rabbit IgG (HRP) (ab97080; diluted 50000 times), and signal was developed by TMB substrate.



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

This image is courtesy of an Abreview submitted by Kirk Mcmanus.

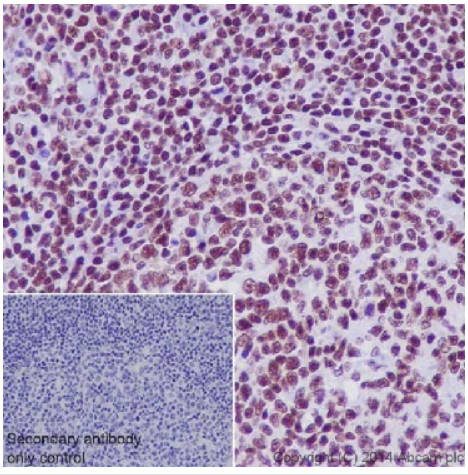
ab5095 staining RNA polymerase II CTD repeat YSPTSPS (phospho S2) in HeLa cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X100. Samples were incubated with primary antibody (1/200 in PBS) for 1 hour at 22°C. An Alexa Fluor® 488 conjugated Goat Polyclonal Anti-rabbit (1/200) was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab5095 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on epithelium cells and glomerulus cells of mouse kidney was observed. Counter stained with Hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH9.

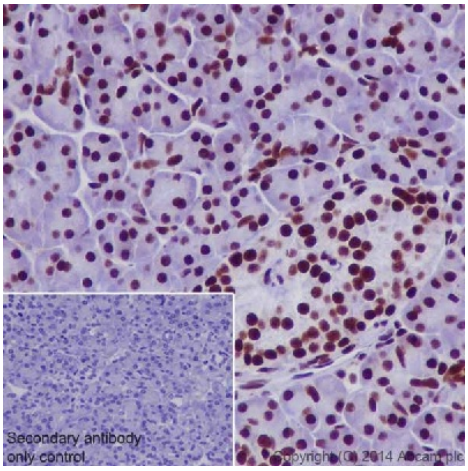
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab5095 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on human tonsil was observed. Counter stained with Hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH9.

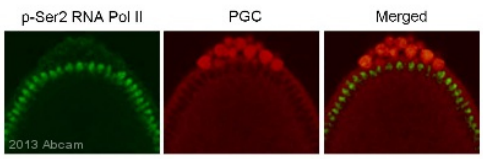
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

Immunohistochemical analysis of paraffin-embedded Human pancreas tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab5095 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on epithelium cells and pancreas islet cells of human pancreas was observed. Counter stained with Hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH9.

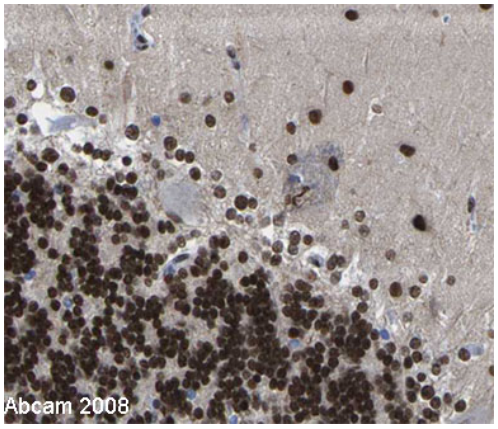
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.



IHC - Wholemount - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

This image is courtesy of an anonymous Abreview

IHC - Wholemount of Caenorhabditis elegans larvae labelling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab5095. The sample was incubated with primary antibody (1/500 in PBS + 3% BSA + 0.1% Triton X-100) for 12 hours at 4°C. ab150077, an goat anti-rabbit Alexa Fluor® 488 (1/1000), was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody - ChIP Grade (ab5095)

Image courtesy of Human Protein Atlas

ab5095 staining in human brain, showing staining of the Purkinje cells (in brown). Paraffin embedded brain tissue was incubated with ab5095 (1:900 dilution) for 30 mins at room temperature. Antigen retrieval was performed by heat induction in citrate buffer pH 6. ab5095 was tested in a tissue microarray (TMA) containing a wide range of normal and cancer tissues as well as a cell microarray consisting of a range of commonly used, well characterised human cell lines. Further results for this antibody can be found at www.proteinatlas.org.

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