abcam

Product datasheet

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

46 Abreviews 494 References 11 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody
Description	Rabbit polyclonal to RNA polymerase II CTD repeat YSPTSPS (phospho S2)
Host species	Rabbit
Specificity	From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help. 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, WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human, Saccharomyces cerevisiae **Predicted to work with:** Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, a wide range of other species
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab12793**)
Positive control	ICC/IF: HeLa cells; NIH-3T3 cells.
General notes	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

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

The Abpromise guarantee 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.

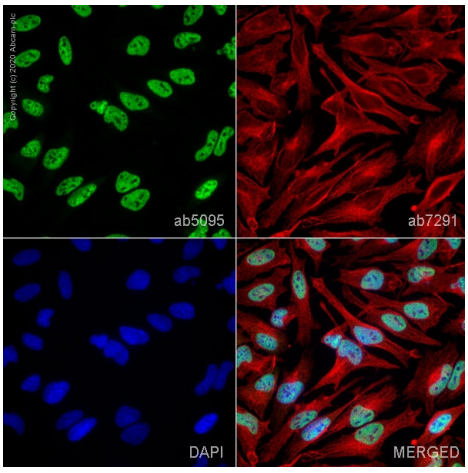
Application	Abreviews	Notes
ELISA	★★★★☆ (1)	Use at an assay dependent concentration.
WB	★★★★☆ (17)	Use a concentration of 1 µg/ml. Predicted molecular weight: 217 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★☆ (5)	Use a concentration of 1 µg/ml.

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.

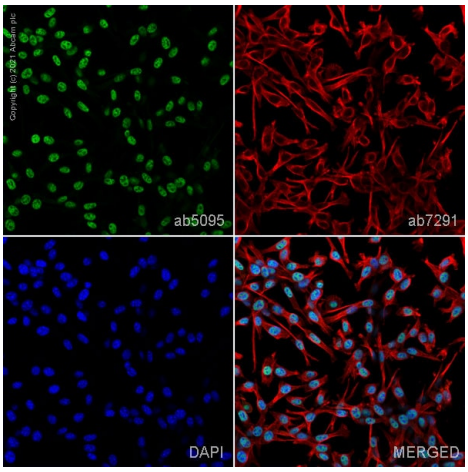
Images



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

ab5095 staining RNA polymerase II CTD repeat YSPTSPS (phospho S2) in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 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 ab5095 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed 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 red). Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 100% methanol (5 min).

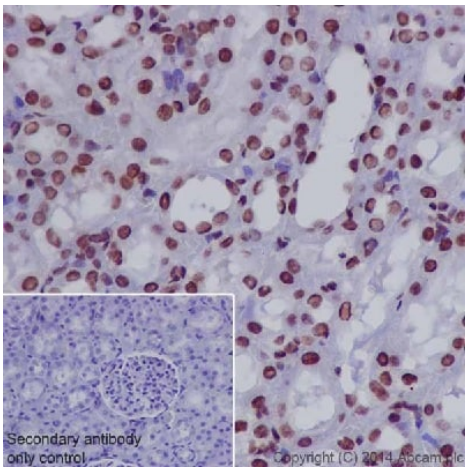
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



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

ab5095 staining RNA polymerase II CTD repeat YSPTSPS (phospho S2) in NIH-3T3 cells. The cells were fixed with 4% paraformaldehyde (10 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 ab5095 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

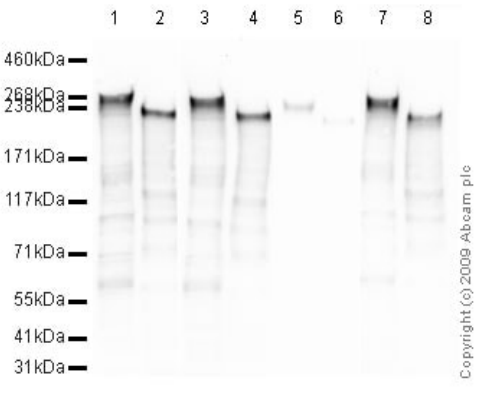
Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



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



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

All lanes : Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody (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 S2) peptide (**ab12793**) at 1 µg/ml
Lane 6 : S.cerevisiae (Y190) Whole Cell Lysate with S. cerevisiae RNA polymerase II CTD repeat YSPTSPS (phospho S2) 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

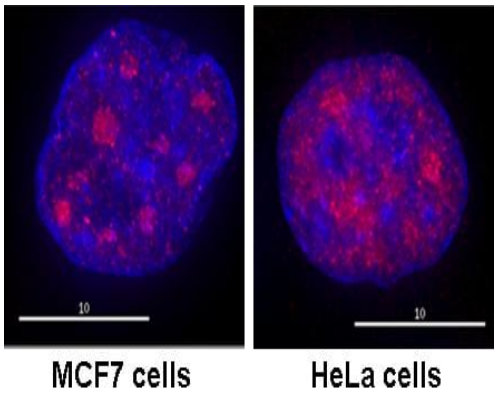
Lysates/proteins at 10 µg per lane.

Secondary

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

Performed under reducing conditions.

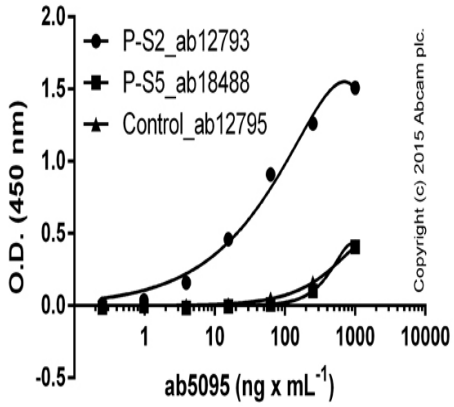
Predicted band size: 217 kDa
Observed band size: 240 kDa



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

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

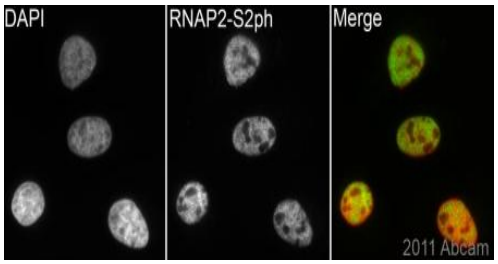
HeLa or MCF7 cells were fixed with 4% formaldehyde in PEM buffer. The coverslip was incubated in blocking buffer of 5% powdered milk in TBS-T plus 0.02% sodium azide for 1 hour at room temperature. Blocking buffer was removed and primary antibody was added at a dilution of 1/500 and incubated overnight 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)

ab12793 - *S. cerevisiae* RNA polymerase II CTD repeat YSPTSPS (phospho S1606 + S1613) peptide
ab18488 - Human RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide

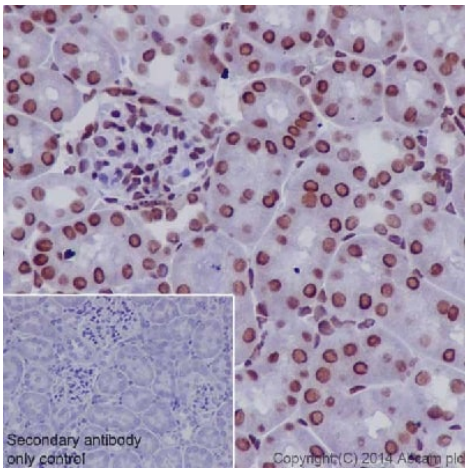
Diluted ab5095 was bound to immobilised phospho- or control peptides (1 microgram per mL). The antibody was detected by goat anti-rabbit IgG (HRP) (**ab97080**; diluted 50000 times), and signal was developed by TMB substrate.



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

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

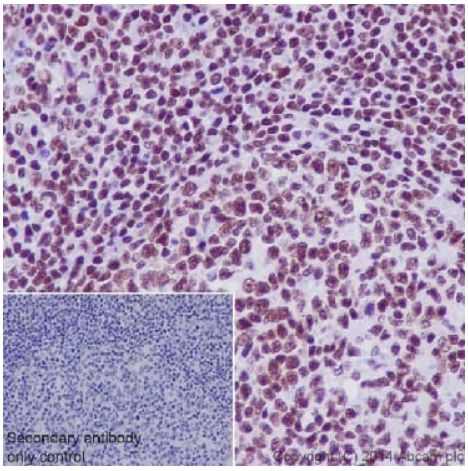
ab5095 staining RNA polymerase II CTD repeat YSPTSPS (phospho S2) in HeLa cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X100. Samples were incubated with primary antibody (1/200 in PBS) for 1 hour at 22°C. An Alexa 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.

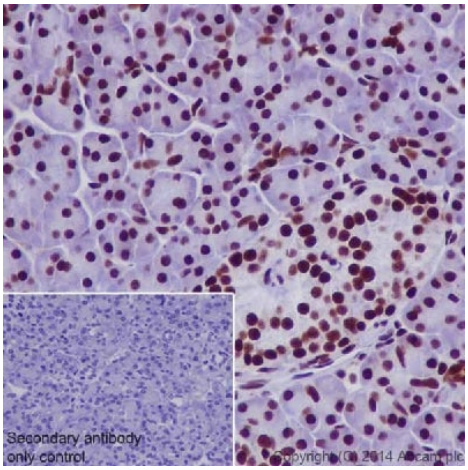
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.



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.

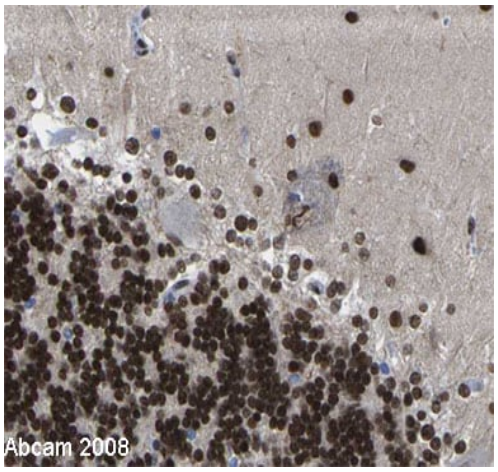
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.



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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody (ab5095)
Image courtesy of Human Protein Atlas

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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