abcam

ab5408

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) is a mouse monoclonal antibody for **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, ELISA, ChIP, Dot Blot**. Suitable for **Human, Mouse, Rat**.

- Over 360 publications
- Trusted since 2003

Recombinant

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	4H8
Purification technique	Affinity purification Protein A
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIP

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

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

Predicted

Species	Arabidopsis thaliana
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

Expected

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

Predicted

Species	Arabidopsis thaliana
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

I-ELISA

Tested

Species	Synthetic peptide - Human
Dilution info	0.25 µg/mL
Notes	-

Expected

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

Predicted

Species	Arabidopsis thaliana
Dilution info	-

Notes	-

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	1/1000
Notes	-

Expected

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

Predicted

Species	Arabidopsis thaliana
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000

Notes	-

Predicted

Species	Arabidopsis thaliana
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500
Notes	-

Expected

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

Predicted

Species	Arabidopsis thaliana
Dilution info	-

Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Target data

See full target information POLR2A pS5

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 duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) is a mouse monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF), ELISA, ChIP, Dot Blot in Human, Mouse, Rat samples.

What is the molecular weight of RNA polymerase II CTD repeat YSPTSPS?
Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) [4H8] - ChIP Grade (ab5408) specifically detects a band for RNA polymerase II CTD repeat YSPTSPS (UniProt: P24928) at a molecular weight of 217kDa.

Trusted by the scientific community
Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) [4H8] - ChIP Grade (ab5408) was first used in a scientific publication in 2003 and has been cited over 360 times in peer-reviewed journals.

Reviewed by scientists
Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) [4H8] - ChIP Grade (ab5408) has over 20 independent reviews from customers.

Other related products
We have a range of other formats of antibody clone [4H8] also available for your convenience: ab5408, Carrier free - ab264109

The production method has been switched from hybridoma to recombinant on 24th Oct 2025.

Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

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.

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

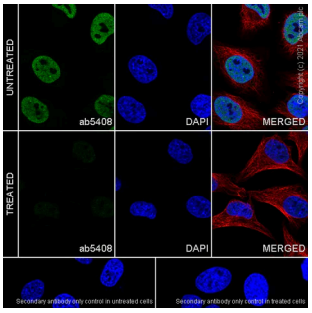
We do not recommend this combination. It is not covered by our product promise.

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.

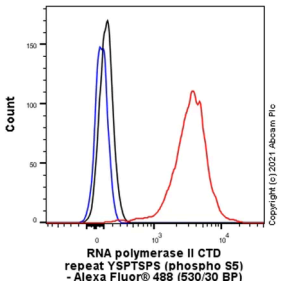
12 product images



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Immunocytochemistry/ Immunofluorescence staining of HeLa cells using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

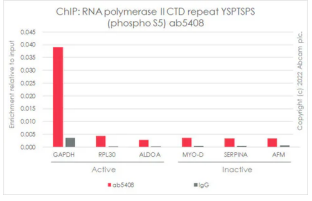
Immunofluorescent analysis of 4% Paraformaldehyde fixed, 0.1% Triton X-100 permeabilized HeLa cells with ab5408 at 1/500 dilution followed by ab150113 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). ab206369 Anti-beta Tubulin rabbit monoclonal antibody (Alexa Fluor® 594) was used for counterstain at a 1/100 dilution (Red). Nuclear DNA was labelled with DAPI (shown in blue)
Confocal image showing nuclear staining in HeLa cell line, the signal decreased after phosphatase treatment at 37°C for 2h.



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

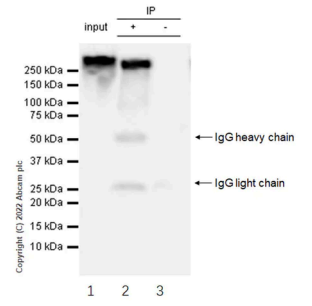
RNA polymerase II CTD repeat YSPTSPS (phospho S5) Flow Cytometry (Intracellular) staining of HeLa (human cervix adenocarcinoma epithelial cell) using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling RNA polymerase II CTD repeat YSPTSPS (phospho S5) with ab5408 at 1/1000 dilution (0.1ug) followed by Goat anti-Mouse IgG (Alexa Fluor® 488, ab150113) secondary at 1/2000 dilution (Red) compared with a Mouse monoclonal IgG isotype control (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).



ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

Chromatin was prepared from HeLa cells according to the Abcam Dual-X-ChIP protocol. Cells were fixed with formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab5408 (red), or 5 µg of Mouse IgG1 ab18443 (gray) and 25 µl of Protein A/G Dynabeads. 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.



Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) was immunoprecipitated from HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab5408 at 1/50 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab5408 at 1/1000 dilution.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:

Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/50 dilution

Lane 1:
HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

Lane 2:
HeLa whole cell lysate

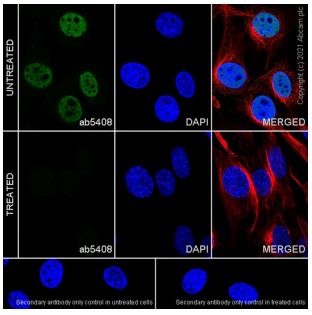
Lane 3:
Mouse IgG1, kappa monoclonal isotype control (ab184443) instead of ab5408 in HeLa whole cell lysate

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Predicted band size: 240 kDa

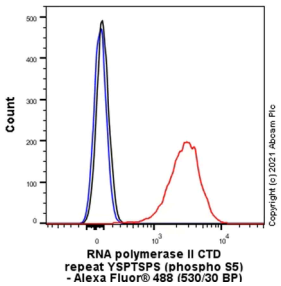
Exposure time: 10s



Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Immunocytochemistry/ Immunofluorescence staining of NIH/3T3 cells using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

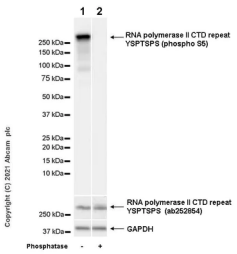
Immunofluorescent analysis of 4% Paraformaldehyde fixed, 0.1% Triton X-100 permeabilized NIH/3T3 cells with ab5408 at 1/500 dilution followed by ab150113 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). ab206369 Anti-beta Tubulin rabbit monoclonal antibody (Alexa Fluor® 594) was used for counterstain at a 1/100 dilution (Red). Nuclear DNA was labelled with DAPI (shown in blue)
Confocal image showing nuclear staining in NIH/3T3 cell line, the signal decreased after phosphatase treatment at 37°C for 2h.



Flow Cytometry (Intracellular) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Flow Cytometry (Intracellular) staining of NIH/3T3 (mouse embryonic fibroblast) using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling RNA polymerase II CTD repeat YSPTSPS (phospho S5) with ab5408 at 1/1000 dilution (0.1ug) followed by Goat anti-Mouse IgG (Alexa Fluor® 488, ab150113) secondary at 1/2000 dilution (Red) compared with a Mouse monoclonal IgG isotype control (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Western blot staining using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

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: 23071310)
All lanes:
Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/1000 dilution

Lane 1:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate (Untreated membrane) at 10 µg

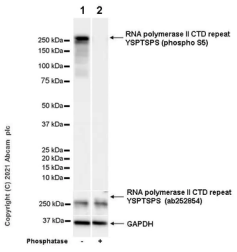
Lane 2:
MCF7 whole cell lysate (Phosphatase treated membrane) at 10 µg

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Predicted band size: 260 kDa

Exposure time: 3.25s



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Western blot staining using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

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: 23071310)
All lanes:
Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/1000 dilution

Lane 1:
PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate (Untreated membrane) at 10 µg

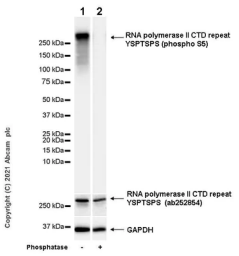
Lane 2:
PC-12 whole cell lysate (Phosphatase treated membrane) at 10 µg

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Predicted band size: 260 kDa

Exposure time: 15s



Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Western blot staining using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

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: 23071310)
All lanes:
Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/1000 dilution

Lane 1:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate (Untreated membrane) at 10 µg

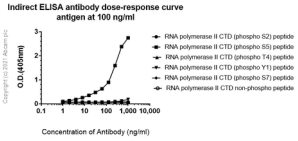
Lane 2:
NIH/3T3 whole cell lysate (Phosphatase treated membrane) at 10 µg

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Predicted band size: 260 kDa

Exposure time: 3.25s

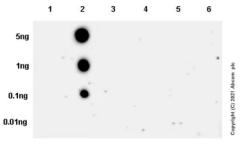


Indirect ELISA - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

Indirect ELISA analysis of ab5408 at 1000-0 ng/ml. The Secondary antibody used was Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) at 1/1000 dilution.

Antigen: RNA polymerase II CTD (phospho S2) peptide, phospho S5 peptide, phospho T4 peptide, phospho Y1peptide, phospho S7 peptide, non-phospho peptide.

Antigen concentration: 100 ng/ml



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) Dot Blot staining using mouse Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody

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

All lanes:
Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408) at 1/1000 dilution

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

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Predicted band size: 260 kDa

Exposure time: 3min

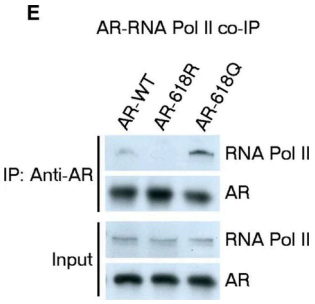


Image collected and cropped by CiteAb under a CC-BY license from the publication

Western blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade (ab5408)

RNA polymerase II CTD repeat YSPTSPS (phospho S5) western blot using anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody [4H8] - ChIP Grade ab5408. Publication image and figure legend from DePaolo, J. S., Wang, Z., et al., 2016, Oncotarget, PubMed 27659526.

ab5408 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab5408 please see the product overview.

ARD1-dependent acetylation enhances AR transcriptional activity(A) Luciferase reporter assays in DU 145 cells co-transfected with PSA-Luc reporter with AR-WT, AR-618R, AR-618Q, or empty vector respectively. Results presented as mean of three replicates ± SD. (B and C) Differential binding of AR-WT, -618R, or -618Q to PSA and TMPRSS2 promoters by ChIP assays using anti-AR antibodies. Normalized relative densitometry was shown above. (D) Quantification of in vivo expression levels of mRNA for TMPRSS2 (AR positively-regulated) and UGT2B15 (AR negatively-regulated) in DU 145 cells stably expressing AR-WT, AR-618R, AR-618Q, or empty vector by qRT-PCR. Values represent the mean of triplicate experiments ± SD. (E) Immunoblot of co-immunoprecipitated RNA polymerase II by transiently-transfected AR-WT, AR-618R, or AR-618Q in DU 145 cells.

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