

Phospho-Rpb1 CTD (Ser5) Antibody



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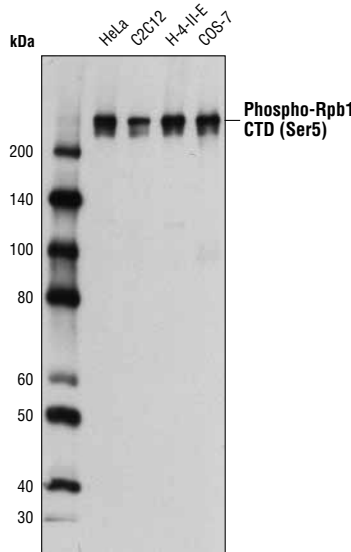
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For Research Use Only. Not For Use In Diagnostic Procedures.

Applications W Endogenous	Species Cross-Reactivity* H, M, R, Mk, (Hm, Dm, X, Z, B, Sc, Ce)	Molecular Wt. 250 kDa	Source Rabbit**
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Background: RNA polymerase II (RNAPII) is a large multi-protein complex that functions as a DNA-dependent RNA polymerase, catalyzing the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates (1). The largest subunit, RNAPII subunit B1 (Rpb1), also known as RNAPII subunit A (POLR2A), contains a unique heptapeptide sequence (Tyr1,Ser2,Pro3,Thr4,Ser5,Pro6,Ser7), which is repeated up to 52 times in the carboxy-terminal domain (CTD) of the protein (1). This CTD heptapeptide repeat is subject to multiple post-translational modifications, which dictate the functional state of the polymerase complex. Phosphorylation of the CTD during the active transcription cycle integrates transcription with chromatin remodeling and nascent RNA processing by regulating the recruitment of chromatin modifying enzymes and RNA processing proteins to the transcribed gene (1). During transcription initiation, RNAPII contains a hypophosphorylated CTD and is recruited to gene promoters through interactions with DNA-bound transcription factors and the Mediator complex (1). The escape of RNAPII from gene promoters requires phosphorylation at Ser5 by CDK7, the catalytic subunit of transcription factor IIH (TFIIH) (2). Phosphorylation at Ser5 mediates the recruitment of RNA capping enzymes, in addition to histone H3 Lys4 methyltransferases, which function to regulate transcription initiation and chromatin structure (3,4). After promoter escape, RNAPII proceeds down the gene to an intrinsic pause site, where it is halted by the negative elongation factors NELF and DSIF (5). At this point, RNAPII is unstable and frequently aborts transcription and disassociates from the gene. Productive transcription elongation requires phosphorylation at Ser2 by CDK9, the catalytic subunit of the positive transcription elongation factor P-TEFb (6). Phosphorylation at Ser2 creates a stable transcription elongation complex and facilitates recruitment of RNA splicing and polyadenylation factors, in addition to histone H3 Lys36 methyltransferases, which function to promote elongation-compatible chromatin (7,8). Ser2/Ser5-phosphorylated RNAPII then transcribes the entire length of the gene to the 3' end, where transcription is terminated. RNAPII dissociates from the DNA and is recycled to the hypophosphorylated form by various CTD phosphatases (1).

In addition to Ser2/Ser5 phosphorylation, Ser7 of the CTD heptapeptide repeat is also phosphorylated during the active transcription cycle. Phosphorylation at Ser7 is required for efficient transcription of small nuclear (sn) RNA genes (9,10). snRNA genes, which are neither spliced nor polyadenylated, are structurally different from protein-coding genes. Instead of a poly(A) signal found in protein-coding



Western blot analysis of extracts from various cell lines using Phospho-Rpb1 CTD (Ser5) Antibody.

RNAs, snRNAs contain a conserved 3'-box RNA processing element, which is recognized by the Integrator snRNA 3' end processing complex (11,12). Phosphorylation at Ser7 by CDK7 during the early stages of transcription facilitates recruitment of RPAP2, which dephosphorylates Ser5, creating a dual Ser2/Ser7 phosphorylation mark that facilitates recruitment of the Integrator complex and efficient processing of nascent snRNA transcripts (13-15).

Specificity/Sensitivity: Phospho-Rpb1 CTD (Ser5) Antibody recognizes endogenous levels of Rpb1 only when the carboxy-terminal domain (CTD) heptapeptide repeat [Tyr1,Ser2,Pro3,Thr4,Ser5,Pro6,Ser7] is phosphorylated at Ser5. This antibody does not cross-react with Rpb1 CTD phosphorylated at Ser2 or Ser7.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser5 of the Rpb1 CTD heptapeptide repeat. Antibodies are purified by protein A and peptide affinity chromatography.

Entrez-Gene ID #5430
Swiss-Prot Acc. #P24928

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

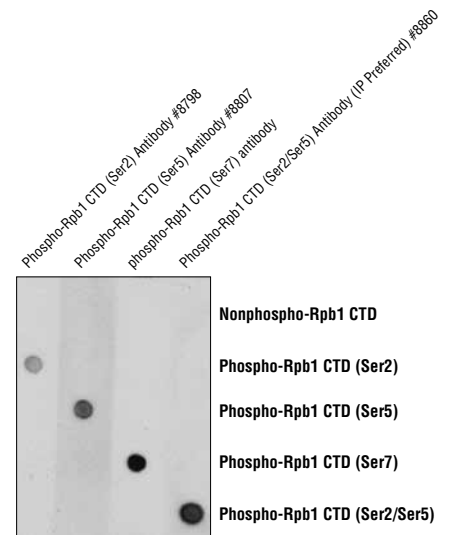
**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting 1:1000

For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended complementary products.



Phospho-Rpb1 CTD antibody specificity is demonstrated using peptide dot blot. Antibody binding to pre-coated Rpb1 CTD peptides is shown using Phospho-Rpb1 CTD (Ser2) Antibody #8798, Phospho-Rpb1 CTD (Ser5) Antibody #8807, a phospho-Rpb1 CTD (Ser7) antibody, and Phospho-Rpb1 CTD (Ser2/Ser5) Antibody (IP Preferred) #8860. As expected, the Phospho-Rpb1 CTD (Ser5) Antibody #8807 only binds to the phospho-Rpb1 CTD peptide that is phosphorylated at Ser5.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Background References:

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