Revision 6

Store at -20C

#13523

Phospho-Rpb1 CTD (Ser5) (D9N5I) Rabbit mAb

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355) orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, W-S, IP, ChIP, ChIP-seq, C&R, C&T	H M R Mk	Endogenous	250	Rabbit IgG	#P24928	5430

Product Usage Information

For optimal ChIP and ChIP-seq results, use 10 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

The CUT&Tag dilution was determined using CUT&Tag Assay Kit #77552.

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunoprecipitation	1:100
Chromatin IP	1:50
Chromatin IP-seq	1:50
CUT&RUN	1:50
CUT&Tag	1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Phospho-Rpb1 CTD (Ser5) (D9N5I) Rabbit mAb 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.

Species predicted to react based on 100% sequence homology

Hamster, D. melanogaster, Xenopus, Bovine, Pig, S. cerevisiae, C. elegans

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser5 of the human Rpb1 CTD heptapeptide repeat.

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 dissociates 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 poly-adenylated, are structurally different from protein-coding genes. Instead of a poly(A) signal found in protein-coding 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).

Background References

1. Brookes, E. and Pombo, A. (2009) *EMBO Rep* 10, 1213-9.
2. Komarnitsky, P. et al. (2000) *Genes Dev* 14, 2452-60.
3. Ho, C.K. and Shuman, S. (1999) *Mol Cell* 3, 405-11.
4. Ng, H.H. et al. (2003) *Mol Cell* 11, 709-19.
5. Cheng, B. and Price, D.H. (2007) *J Biol Chem* 282, 21901-12.
6. Marshall, N.F. et al. (1996) *J Biol Chem* 271, 27176-83.
7. Krogan, N.J. et al. (2003) *Mol Cell Biol* 23, 4207-18.
8. Proudfoot, N.J. et al. (2002) *Cell* 108, 501-12.
9. Chapman, R.D. et al. (2007) *Science* 318, 1780-2.
10. Egloff, S. et al. (2007) *Science* 318, 1777-9.
11. Egloff, S. et al. (2008) *Biochem Soc Trans* 36, 590-4.
12. Baillat, D. et al. (2005) *Cell* 123, 265-76.
13. Akhtar, M.S. et al. (2009) *Mol Cell* 34, 387-93.
14. Egloff, S. et al. (2010) *J Biol Chem* 285, 20564-9.
15. Egloff, S. et al. (2012) *Mol Cell* 45, 111-22.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

W: Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq **C&R:** CUT&RUN **C&T:** CUT&Tag

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Mk:** Monkey

Trademarks and Patents

Limited Uses