Revision 1

Store at -20C

#54020

Rpb1 CTD Antibody Sampler Kit

1 Kit (5 x 20 microliters)

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Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
Phospho-Rpb1 CTD (Ser2) (E1Z3G) Rabbit mAb	13499	20 µl	250 kDa	Rabbit IgG
Phospho-Rpb1 CTD (Ser5) (D9N5I) Rabbit mAb	13523	20 µl	250 kDa	Rabbit IgG
Phospho-Rpb1 CTD (Ser2/Ser5) (D1G3K) Rabbit mAb	13546	20 µl	250 kDa	Rabbit IgG
Phospho-Rpb1 CTD (Ser7) (E2B6W) Rabbit mAb	13780	20 µl	250 kDa	Rabbit IgG
Rpb1 NTD (D8L4Y) Rabbit mAb	14958	20 µl	250 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

Description

The Rpb1 CTD Antibody Sampler Kit provides an economical means of evaluating total Rpb1 NTD levels as well as Rpb1 CTD phosphorylated and specific sites. The kit includes enough primary antibodies to perform two western blot experiments per primary antibody.

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.

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.

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