

Store at
-20C
#64828**SPT4 (D3P2W) Rabbit mAb**

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Applications: W, IP, ChIP, C&R, C&T	Reactivity: H M R Mk	Sensitivity: Endogenous	MW (kDa): 13	Source/Isotype: Rabbit IgG	UniProt ID: #P63272	Entrez-Gene Id: 6827
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Product Usage Information

For optimal ChIP results, use 5 µl of antibody and 10 µg of chromatin (approximately 4 x 10⁶ 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
Immunoprecipitation	1:50
Chromatin IP	1:100
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

SPT4 (D3P2W) Rabbit mAb recognizes endogenous levels of total SPT4 protein.

Species predicted to react based on 100% sequence homology

Hamster, *S. cerevisiae*

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human SPT4 protein.

Background

DRB-sensitivity inducing factor (DSIF), a heterodimer composed of SPT4 and SPT5, is capable of both facilitating and inhibiting RNA polymerase II (RNAPII) activity (1-3). DSIF, together with NELF (Negative Elongation Factor), inhibits RNAPII elongation, resulting in promoter proximal pausing of RNAPII as it awaits additional signaling to resume transcription (4). The release of promoter proximal pausing is signaled through phosphorylation of the RNAPII C-terminal domain (CTD) and NELF by positive transcription elongation factor (P-TEFb) (5). P-TEFb also phosphorylates SPT5 at Thr4 within the evolutionarily conserved heptapeptide repeat motif. This phosphorylation event switches DSIF from a transcriptional repressor to an activator where it becomes a critical factor for transcriptional elongation (6,7).

Background References

1. Wada, T. et al. (1998) *Genes Dev* 12, 343-56.
2. Saunders, A. et al. (2006) *Nat Rev Mol Cell Biol* 7, 557-67.
3. Gomes, N.P. et al. (2006) *Genes Dev* 20, 601-12.
4. Yamaguchi, Y. et al. (1999) *Cell* 97, 41-51.
5. Buratowski, S. (2009) *Mol Cell* 36, 541-6.
6. Wada, T. et al. (1998) *EMBO J* 17, 7395-403.
7. Yamada, T. et al. (2006) *Mol Cell* 21, 227-37.

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 **IP:** Immunoprecipitation **ChIP:** Chromatin IP **C&R:** CUT&RUN **C&T:** CUT&Tag

Cross-Reactivity Key

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

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