

Phospho-Histone H3 (Thr3) (D5G1I) Rabbit mAb

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

Applications: W, IF-IC, FC-FP, ChIP	Reactivity: H M	Sensitivity: Endogenous	MW (kDa): 17	Source/Isotype: Rabbit IgG	UniProt ID: #P68431	Entrez-Gene Id: 8350
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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.

Application

Western Blotting
Immunofluorescence (Immunocytochemistry)
Flow Cytometry (Fixed/Permeabilized)
Chromatin IP

Dilution

1:1000
1:3200
1:200
1:100

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.

For a carrier free (BSA and azide free) version of this product see product #81499.

Specificity/Sensitivity

Phospho-Histone H3 (Thr3) (D5G1I) Rabbit mAb recognizes endogenous levels of histone H3 protein only when phosphorylated at Thr3.

Species predicted to react based on 100% sequence homology

Rat, Zebrafish, *S. cerevisiae*

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr3 of human histone H3 protein.

Background

Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3, and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various posttranslational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (2-5). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, gene expression (6). In most species, histone H2B is primarily acetylated at Lys5, 12, 15, and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18, 23, 27, and 56. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms (2,3). Phosphorylation at Ser10, Ser28, and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8-10). Phosphorylation at Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation at Thr3 of H3 in prophase and its dephosphorylation during anaphase (11).

Background References

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3. Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41-5.
4. Cheung, P. et al. (2000) *Cell* 103, 263-71.
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6. Jaskelioff, M. and Peterson, C.L. (2003) *Nat Cell Biol* 5, 395-9.
7. Thorne, A.W. et al. (1990) *Eur J Biochem* 193, 701-13.
8. Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-60.
9. Goto, H. et al. (1999) *J Biol Chem* 274, 25543-9.
10. Preuss, U. et al. (2003) *Nucleic Acids Res* 31, 878-85.
11. Dai, J. et al. (2005) *Genes Dev* 19, 472-88.

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 **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP

Cross-Reactivity Key **H:** Human **M:** Mouse

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