

## TECHNICAL DATASHEET

# H3T3pK4me2 polyclonal antibody - Classic

Cat. No. C15410278	Specificity: Human, mouse, C. elegans, rat, chicken,	
Type: Polyclonal	Xenopus, Drosophila, plant	
Source: Rabbit	Purity: Affinity purified	
Lot #: 001	<b>Storage:</b> Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.	
<b>Size:</b> 50 µg		
Concentration: 0.83 µg/µl	<b>Precautions:</b> This product is for research use only. Not for use in diagnostic or therapeutic procedures.	

### **Applications**

	Suggested dilution	Results
ChIP	2-5 µg/million cells	Figure 1
Immunohistochemistry	1:50	
IF	1:50	Figure 2
Western blot	1:500	Figure 3
Dot blot	1:1,000	Figure 4

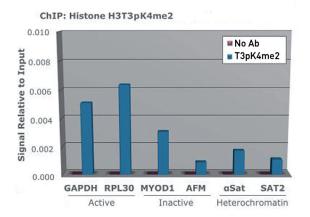
### Target description

Chromatin is the arrangement of DNA and proteins in which chromosomes are formed. Correspondingly, chromatin is formed from nucleosomes, which are comprised of a set of four histone proteins (H2A, H2B, H3, H4) wrapped with DNA. Chromatin is a very dynamic structure in which numerous post-translational modifications work together to activate or repress the availability of DNA to be copied, transcribed, or repaired. These marks decide which DNA will be open and commonly active (euchromatin) or tightly wound to prevent access and activation (heterochromatin). Common histone modifications include methylation of lysine and arginine, acetylation of lysine, phosphorylation of threonine and serine, and sumoylation, biotinylation, and ubiquitylation of lysine. Specifically, the dimethylation of H3 lysine 4 (H3 K4Me2) and phosphorylation of threonine 3 (H3T3p) are known marks of transcriptional activation and mitosis, respectively. While H3K4 has many known modifying enzymes (Set1, Set7/9, MLL, ASH1), Haspin is the only known modifier for H3T3. Recent findings also demonstrate that T3p can promote binding of survivin in the nucleosome.



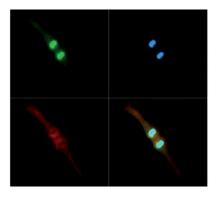
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### Results



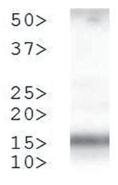
#### Figure 1. H3T3pK4me2 antibody ChIP results

Chromatin Immunoprecipitation of H3T3pK4me2 antibody. Chromatin from one million formaldehyde cross-linked Hela cells was used with 2  $\mu$ g of H3T3pK4me2 and 20  $\mu$ l of magnetic beads per immunoprecipitation. A no antibody (No Ab) control was also used. Immunoprecipitated DNA was quantified using quantitative real-time PCR and normalized to the input chromatin.



#### Figure 2. H3T3pK4me2 antibody Immunofluorescence results

Immunofluorescence H3T3pK4me2 antibody. Tissue: HeLa cells. Fixation: 0.5% PFA. Primary antibody was used at a 1:50 dilution for 1 h at RT. Secondary antibody: Dylight 488 secondary antibody at 1:10,000 for 45 min at RT. Localization: Histone H3T3pK4me2 is nuclear and chromosomal. Staining: H3T3pK4me2 is expressed in green while the nuclei and aplpha-tubulin were coexpressed with DAPI (blue) and Dylight 550 (red).

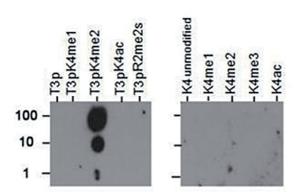


#### Figure 3. H3T3pK4me2 antibody Western blot results

Western Blot of H3T3pK4me2 antibody. 30 µg of NIH-3T3 histone extracts. Primary antibody used at a 1:500 dilution overnight at 4°C. Secondary antibody: IRDye800<sup>™</sup> rabbit secondary antibody at 1:10,000 for 45 min at RT. Predicted/Observed size: ~15 kDa. Other band(s): None.



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#### Figure 4. H3T3pK4me2 antibody Dot blot results

Dot Blot of H3T3pK4me2 antibody. Load: 1, 10, and 100 picomoles of the different peptides. Primary antibody used at a 1:1,000 dilution for 45 min at 4°C. Secondary antibody: Dylight™488 rabbit secondary antibody at 1:10,000 for 45 min at RT.

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