BACKGROUND
Post-translation modifications of histones modulate the accessibility and transcriptional competence of specific chromatin regions within the eukaryotic genome. Phosphorylation of histone H3 is unique in the sense that it associates on one hand with open chromatin during gene activation and marks on the other hand highly condensed chromatin during mitosis.

Product type: Primary antibodies
Immunogen: Synthetic peptide corresponding to N-terminus region Thr32ph (aa 21-39) of human Histone H3, ATKAARKSAPS(phT)GGVKKPH
Rased in: Rat
Myeloma: SP2
Clone number: 6C7G12
Isotype: IgG2a, κ
Host: -
Source: Culture supernatant
Purification: Ion-exchange chromatography
Form: Liquid
Storage buffer: PBS containing 50% Glycerol, 0.05% NaN₃ as a preservative
Concentration: 1 mg / ml
Volume: 100 ul
Label: Unlabeled
Specificity: Histone H3 T32ph Epitope : phosphorylated Thr32 of Histone H3
Cross reactivity: Human, Monkey, Mouse, Rat, Hamster
Other species have not been tested.
Storage: Store below -20°C (below -70°C for prolonged storage)
Aliquot to avoid cycles of freeze/thaw.
Other: Data Link : UniProtKB/Swiss-Prot P68431
* recommended positive controls is mammalian cell

Application notes
Recommended dilutions
Western blotting: 1/1000 – 1/5000
Immunocytochemistry: 1/100 -1/500

Other applications have not been tested.
Optimal dilutions/concentrations should be determined by the end user.

References
1) Strahl and Allis, Nature. 2000 Jan 6;403(6765):41-5. PMID: 10638745

www.cosmobio.com
ANTIBODY CHARACTERIZATION

Fig. 1  The composition of Histone H3 peptides and the reactivity of Histone H3 T32ph antibody, 6C7G12.

![Graph showing the composition of Histone H3 peptides and reactivity of 6C7G12 antibody]

<table>
<thead>
<tr>
<th>H3 T32ph (6C7G12)</th>
<th>H3.1</th>
<th>21 ATKAARKS APAT GGVKKPH 39</th>
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<tr>
<td>H3.1 S28ph</td>
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<td>H3.3</td>
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</tr>
<tr>
<td>H3 T11ph</td>
<td>1 ARTKQTARKSTphGGKAPRKQC 19</td>
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Fig. 2  Western blot analysis of the treated-cell extracts using Histone H3 T32ph antibody, 6C7G12

![Western blot analysis showing the expression of Histone H3 T32ph in treated cells]

<table>
<thead>
<tr>
<th>HeLa</th>
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<tr>
<td>6C7G12</td>
<td>+ +</td>
</tr>
<tr>
<td>Control H3</td>
<td>- -</td>
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</table>

HeLa Cells were treated before and after UV irradiation.

1: After 12 hours from released Nocodazole.
2: Before UV irradiation
3: After UV irradiation (100 J/m², 2 hours)

Fig. 3  Immunocytochemical analysis of HeLa Cell using Histone H3 T32ph antibody, 6C7G12.

![Immunocytochemical analysis showing HeLa interphase and metaphase]

**RELATED PRODUCTS:**

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Clone</th>
<th>Application</th>
<th>Maker</th>
<th>Cat#</th>
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<td>CAC</td>
<td>CE-039A</td>
</tr>
<tr>
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<td>ChIP/ WB/ IC/ IHC/ IP</td>
<td>CAC</td>
<td>CE-039B</td>
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<td>CAC</td>
<td>CE-040A</td>
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<td>Anti Histone H3.3 (Variant) Monoclonal Antibody</td>
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<td>CAC</td>
<td>CE-040B</td>
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<tr>
<td>Anti Histone H3 S10ph Monoclonal Antibody</td>
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*For research use only, Not for diagnostic use*
ヒストン H3 バリアントモノクローナル抗体

Anti Histone H3.1/H3.2 [Clone: 6G3C7]
Anti Histone H3.1/H3.2 [Clone: 1D4F2]
Anti Histone H3.3 [Clone: 6C4A3]
Anti Histone H3.3 [Clone: 4H2D7]

抗体の特異性を ELISA 法で確認

H3.3 抗体での実験例

E5 細胞、幹細胞など特定の組織に変化する能力（分化能）を持った細胞は、あらかじめ H3.3 と呼ばれる特殊なヒストン（ヒストンバリアント）により、必要な遺伝子がマーキングされることが明らかになりました。骨格筋形成時に、転写因子 MyoD が骨格筋遺伝子に選択的に結合し、MyoD に結合したシャベロンである Chd2 タンパク質が H3.3 をゲノムに組み込むことでマーキングしていることが発見されました。一方で、マーキング機構を失った細胞では、骨格筋の形成がせられなかったことから、骨格筋形成を事前に予測できる細胞内のメカニズムが明らかとなった（参考文献 1, 2）。

遺伝子「マーキング」により骨格筋となる

マーキングがある場合

遺伝子を H3.3 でマーキングする

マーキングがない場合

骨格筋形成不了

参考文献

製品詳細

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コスモ・バイオ株式会社

人と科学のステキな未来へ
### Histone H3 N-terminal modifications

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### 注意事項

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Histone Variant Monoclonal Antibodies

Anti Histone H3.1/H3.2 [Clone: 6G3C7]  
Anti Histone H3.1/H3.2 [Clone: 1D4F2]  
Anti Histone H3.3 [Clone: 6C4A3]  
Anti Histone H3.3 [Clone: 4H2D7]

Nucleosomes are composed of four different histone proteins designated H2A, H2B, H3, and H4. In humans, five variants of histone H3 are reported: H3.1, H3.2, H3.3, H3t, and CENP-A. The two major Histone H3 variants, H3.1 and H3.3, are the main variants displaying distinct genomic localization patterns in eukaryotes. Deposition of Histone H3.1 is associated with DNA synthesis during DNA replication and possibly DNA repair, while Histone H3.3 is incorporated independently of DNA synthesis and is the predominant form of H3 found in non-dividing cells. Hence, these new Histone H3 variant monoclonal antibodies offer great utility for dissecting the functional significance of these H3 variants and the molecular mechanisms associated with their deposition.

Recently, it was shown that a genomic gene cluster regulating skeletal myogenesis is marked by H3.3 protein prior to cellular muscle formation and that H3.3 marking of this region enables myogenic gene activation (Ref. 2). These results suggest that monitoring H3.3 marking at specific loci may be useful in the prediction of cell fate. These H3.3 monoclonal antibodies are expected to be useful probes in the field of regenerative medicine.

### Antibody specificity by competition peptide ELISA

#### Histone H3.1/H3.2 MAb (6G3C7)

<table>
<thead>
<tr>
<th>Antibody Concentration (µg/ml)</th>
<th>H3.1 peptide</th>
<th>H3.3 peptide</th>
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</thead>
<tbody>
<tr>
<td>0.001</td>
<td>0.100</td>
<td>0.050</td>
</tr>
<tr>
<td>0.010</td>
<td>0.150</td>
<td>0.075</td>
</tr>
<tr>
<td>0.100</td>
<td>0.200</td>
<td>0.100</td>
</tr>
<tr>
<td>0.200</td>
<td>0.250</td>
<td>0.125</td>
</tr>
<tr>
<td>0.300</td>
<td>0.300</td>
<td>0.150</td>
</tr>
<tr>
<td>0.400</td>
<td>0.350</td>
<td>0.175</td>
</tr>
<tr>
<td>0.500</td>
<td>0.400</td>
<td>0.200</td>
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</table>

#### Histone H3.1/H3.2 MAb (1D4F2)

<table>
<thead>
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<th>Antibody Concentration (µg/ml)</th>
<th>H3.1 peptide</th>
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</thead>
<tbody>
<tr>
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<td>0.050</td>
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<td>0.010</td>
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<td>0.200</td>
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<tr>
<td>0.500</td>
<td>0.400</td>
<td>0.200</td>
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#### Histone H3.3 MAb (4H2D7)

<table>
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### Experimental example

These H3 variant antibodies were essential tools in a first of kind study showing that differentiation specific genes are marked for lineage specific expression by the deposition of Histone H3.3 at the onset of differentiation signaling (Ref. 2).

Reference

Histones are the main protein components of chromatin. To facilitate nuclear packaging and control of gene expression, DNA in chromatin is wound around nucleosome particles composed primarily of the Histones H2A, H2B, H3, and H4. Histone N-terminal regions (histone tails) protrude from the nucleosome core and are subject to a variety of reversible, regulated modifications (including acetylation, phosphorylation, and methylation) influencing transcription and chromatin structure. How such modifications are regulated and how these modifications effect gene expression continues to be an area of intense interest and research. In such studies, chromatin immunoprecipitation (ChIP) is perhaps the most widely used experimental procedure. Due to the inherent variability and limited supply of polyclonal antibodies, well characterized monoclonal antibodies are preferred reagents for ChIP. The versatile set of anti-histone monoclonal antibodies offered here are therefore highly valuable reagents to your lab’s epigenetic toolbox.

**Monoclonal Antibodies to Histone Modifications**

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### Histone H3 N-terminal modifications

- **antiphospho**
- **acetyl**
- **trimethyl**
- **monomethyl**

<table>
<thead>
<tr>
<th>Description</th>
<th>Host</th>
<th>Residue</th>
<th>Modification</th>
<th>Clone</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti Histone H3</td>
<td>Mouse</td>
<td>-</td>
<td>unmodified</td>
<td>MABI0001</td>
<td>ChIP/ WB/ IC</td>
<td>MCA-MAB0001-100-EX</td>
<td>100 µL</td>
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<tr>
<td>Anti Monoacetyl Histone H3</td>
<td>Mouse</td>
<td>K9</td>
<td>monoacetyl</td>
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<td>dimethyl</td>
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<td>ChIP/ WB/ IC</td>
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<tr>
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<td>K9</td>
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<td>CAC-CE-037A</td>
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<td>Anti Acetyl Histone H3 (Lys9)</td>
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<td>acetyl</td>
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<td>ChIP/ WB/ IC</td>
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<td>monomethyl</td>
<td>MABI0026</td>
<td>ChIP/ WB/ IC</td>
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<td>monoacetyl</td>
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<td>monoacetyl</td>
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<td>MABI0033</td>
<td>ChIP/ WB/ IC</td>
<td>MCA-MAB0033-100-EX</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

### Histone H3 phospho Ser10 immunostaining

- **DAPI**
- **Anti-phospho H3S10**
- **DAPI/ phospho H3S10**

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