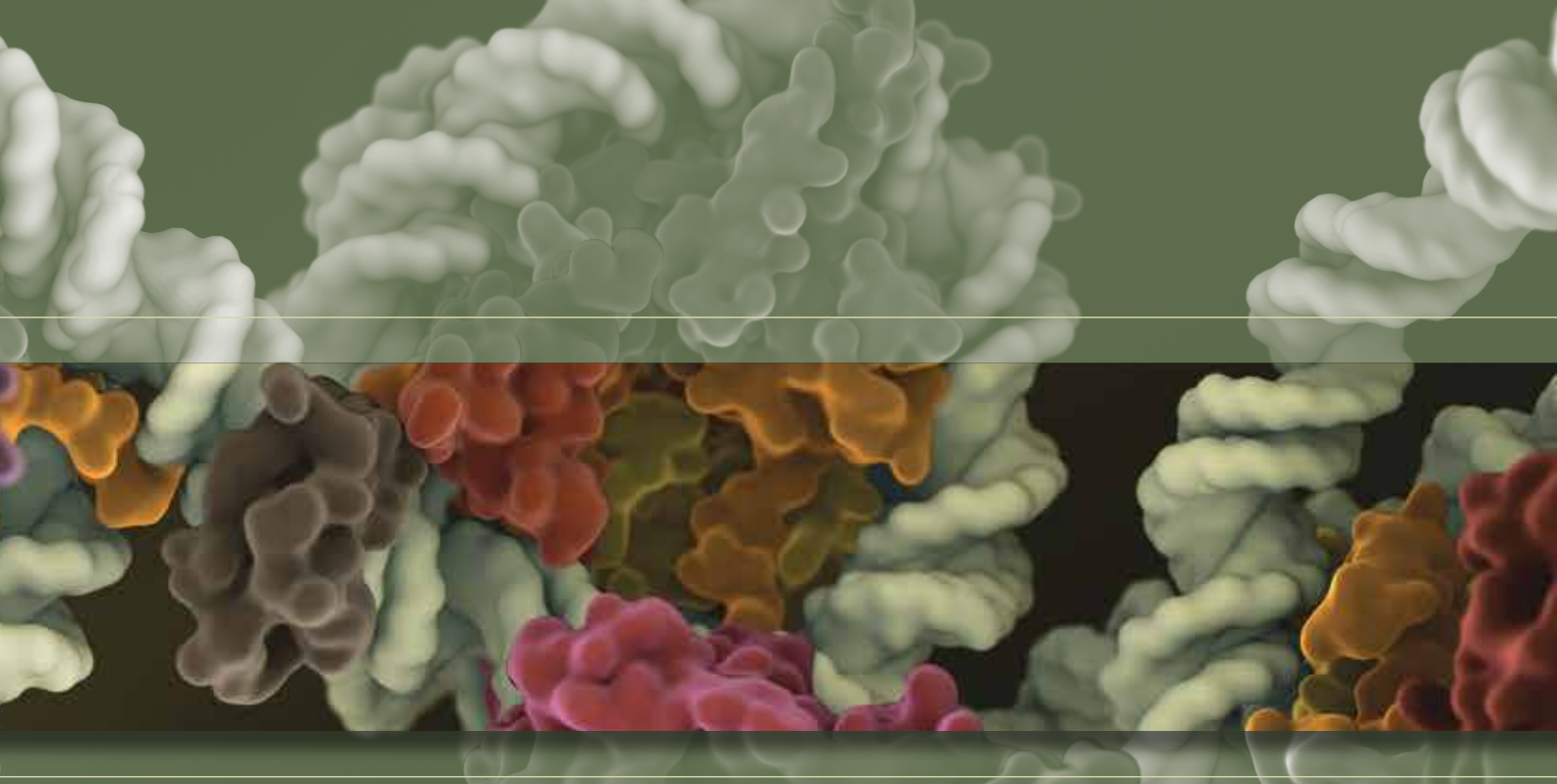

ANTIBODY PERFORMANCE COMPARISON

# Histone Modifications

Cell Signaling TECHNOLOGY®

**COVER IMAGE:** Methylation of cytosine bases in regions called CpG islands is a hallmark of transcriptionally repressed heterochromatin. These methylated cytosines in turn recruit proteins like methyl-CpG binding protein 2 (MeCP2; gray) and heterochromatin protein 1 (HP1; orange). These proteins are thought to maintain a repressive state of chromatin by inducing histone deacetylation by HDACs (purple) as well as histone tail methylation by histone methyltransferase enzymes (red).

## Technical Support

At CST, providing exceptional customer service and technical support are top priorities. Our scientists work at the bench daily to produce and validate our antibodies, so they have hands-on experience and in-depth knowledge of each antibody's performance. In the process, these same scientists generate valuable reference information that they use to answer your questions and help troubleshoot your experiment by phone or email.

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## Contents



# Validation for Histone Modification Antibodies

Antibodies targeted to histone modifications may bind non-specifically to similar, but off-target histone modifications. Conversely, their specific binding can be inhibited by steric hindrance from modifications on neighboring residues. Assays like ELISA, western blot, ChIP, and IF are commonly used to demonstrate antibody specificity and sensitivity, but they cannot clearly predict how an antibody will interact with nearby epitopes. As a result, they are of limited use when trying to validate an antibody to a histone modification target.

For these reasons, the ENCyclopedia Of DNA Elements (ENCODE) Project (National Human Genome Research Institute) established a set of guidelines for the validation of antibodies used for ChIP-seq experiments[1].

CST modification-specific histone antibodies are validated in accordance with the ENCODE guidelines, but we go a step further by using a peptide array assay similar to the one described by Fuchs, S.M., et al[2]. In a single experiment, these arrays assess reactivity against known modifications across all histone proteins as well as the effects of neighboring modifications on the ability of the antibody to detect a single modification site. Therefore, the peptide array assay allows us to confirm the antibodies are performing as expected.

## Array

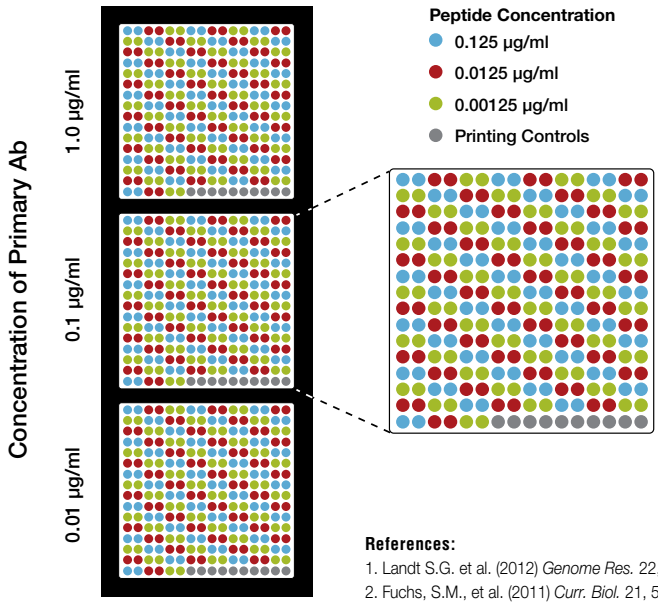
Peptides with mono-,di-, tri-methyl, acetyl-, or unmodified lysine are spotted onto nitrocellulose either alone or in combination with a known neighboring histone modification (e.g., histone H3K4Me3 and H3T3Phos), as indicated in the diagram. A similar array is used for testing methyl-arginine antibodies.

## Antibody

The histone modification antibody is applied to the array at three concentrations, as indicated in the diagram. This allows us to assess antibody reactivity while ensuring that the antibody concentration is not saturating the assay.

## Analysis

The arrays are washed and incubated with a fluorescently tagged secondary antibody and then read using a LI-COR® Odyssey® Infrared Imager.





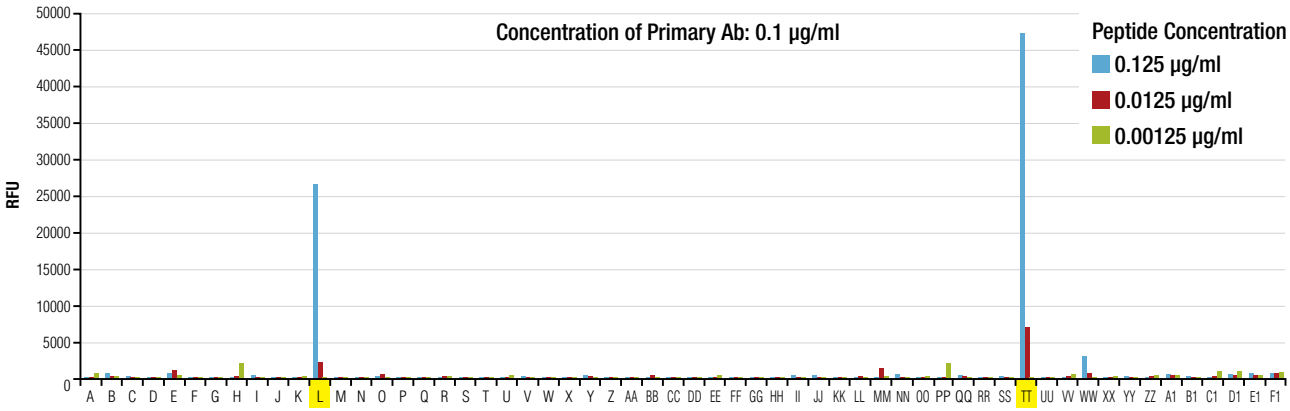
**References:**

1. Landt S.G. et al. (2012) *Genome Res.* 22, 1813–1831.
2. Fuchs, S.M., et al. (2011) *Curr. Biol.* 21, 53–58.

# Tri-Methyl-Histone H3 (Lys27) Rabbit mAb Performance

## Peptide Array

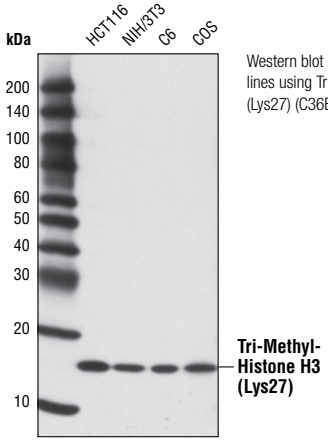
**Tri-Methyl Histone H3 (Lys27) (C36B11) Rabbit mAb #9733** is highly specific for tri-methyl-histone H3 (Lys27) and is not affected by methylation at Arg26.




### Peptide List

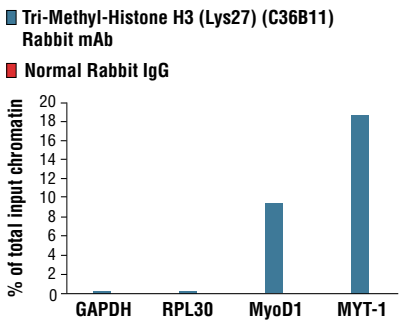
| | |
|---|---|
| **A** | H3 (Lys4) non-methyl |
| **B** | H3 (Lys4) mono-methyl |
| **C** | H3 (Lys4) di-methyl |
| **D** | H3 (Lys4) tri-methyl |
| **E** | H3 (Lys9) non-methyl |
| **F** | H3 (Lys9) mono-methyl |
| **G** | H3 (Lys9) di-methyl |
| **H** | H3 (Lys9) tri-methyl |
| **I** | H3 (Lys27) non-methyl |
| **J** | H3 (Lys27) mono-methyl |
| **K** | H3 (Lys27) di-methyl |
| **L** | H3 (Lys27) tri-methyl |
| **M** | H3 (Lys36) non-methyl |
| **N** | H3 (Lys36) mono-methyl |
| **O** | H3 (Lys36) di-methyl |
| **P** | H3 (Lys36) tri-methyl |
| **Q** | H3 (Lys79) non-methyl |
| **R** | H3 (Lys79) mono-methyl |
| **S** | H3 (Lys79) di-methyl |
| **T** | H3 (Lys79) tri-methyl |
| **U** | H4 (Lys20) non-methyl |
| **V** | H4 (Lys20) mono-methyl |
| **W** | H4 (Lys20) di-methyl |
| **X** | H4 (Lys20) tri-methyl |
| **Y** | H2A (Lys5) non-methyl |
| **Z** | H2A (Lys5) mono-methyl |
| **AA** | H2A (Lys5) di-methyl |
| **BB** | H2A (Lys5) tri-methyl |
| **CC** | H3 (Thr3) phospho/(Lys4) mono-methyl |
| **DD** | H3 (Thr3) phospho/(Lys4) di-methyl |
| **EE** | H3 (Thr3) phospho/(Lys4) tri-methyl |
| **FF** | H3 (Arg2) symmetric-di-methyl/(Lys4) mono-methyl |
| **GG** | H3 (Arg2) symmetric-di-methyl/(Lys4) di-methyl |
| **HH** | H3 (Arg2) symmetric-di-methyl/(Lys4) tri-methyl |
| **II** | H3 (Arg2) asymmetric-di-methyl/(Lys4) mono-methyl |
| **JJ** | H3 (Arg2) asymmetric-di-methyl/(Lys4) di-methyl |
| **KK** | H3 (Arg2) asymmetric-di-methyl/(Lys4) tri-methyl |
| **LL** | H3 (Arg8) symmetric-di-methyl/(Lys9) mono-methyl |
| **MM** | H3 (Arg8) symmetric-di-methyl/(Lys9) di-methyl |
| **NN** | H3 (Arg8) symmetric-di-methyl/(Lys9) tri-methyl |
| **OO** | H3 (Lys9) mono-methyl/(Ser10) phospho |
| **PP** | H3 (Lys9) di-methyl/(Ser10) phospho |
| **QQ** | H3 (Lys9) tri-methyl/(Ser10) phospho |
| **RR** | H3 (Arg26) asymmetric-di-methyl/(Lys27) mono-methyl |
| **SS** | H3 (Arg26) asymmetric-di-methyl/(Lys27) di-methyl |
| **TT** | H3 (Arg26) asymmetric-di-methyl/(Lys27) tri-methyl |
| **UU** | H3 (Lys27) mono-methyl/(Ser28) phospho |
| **VV** | H3 (Lys27) di-methyl/(Ser28) phospho |
| **WW** | H3 (Lys27) tri-methyl/(Ser28) phospho |
| **XX** | H3 (Lys9) mono-methyl/(Ser10/Thr11) phospho |
| **YY** | H3 (Lys9) di-methyl/(Ser10/Thr11) phospho |
| **ZZ** | H3 (Lys9) tri-methyl/(Ser10/Thr11) phospho |
| **A1** | H3 (Thr6) phospho/(Lys9) tri-methyl |
| **B1** | H3 (Lys4) di-methyl/(Thr6) phospho |
| **C1** | H3 (Lys4) mono-methyl/(Thr6) phospho |
| **D1** | H3 (Lys4) tri-methyl/(Thr6) phospho |
| **E1** | H3 (Thr6) phospho/(Lys9) di-methyl |
| **F1** | H3 (Thr6) phospho/(Lys9) mono-methyl |

## Western Blot




Western blot analysis of various cell lines using Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb.

## ChIP

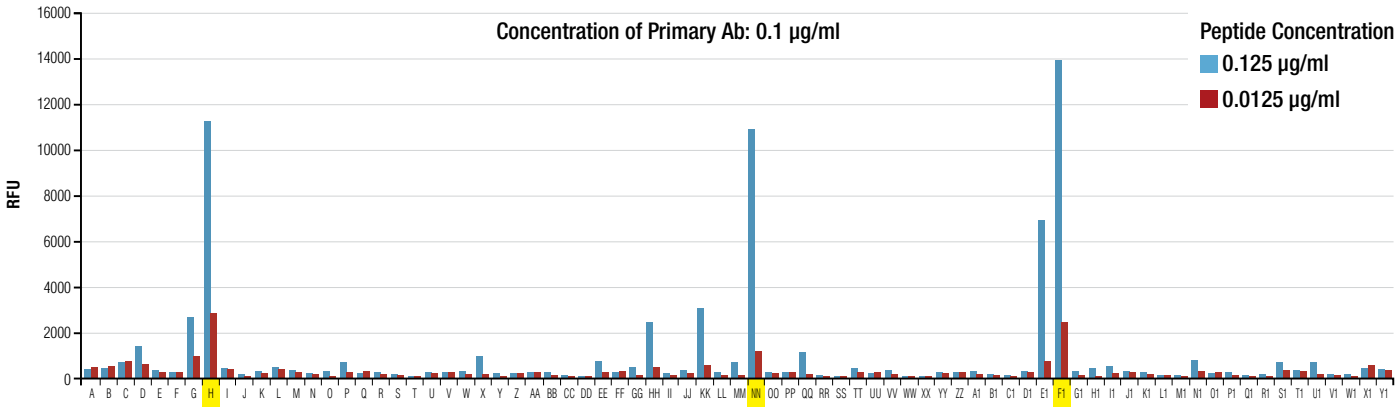



Chromatin immunoprecipitation was performed with crosslinked chromatin from 4 x $10^6$ HeLa cells and either 10 µl of Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb, or 2 µl of Normal Rabbit IgG #2729, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human GAPDH Exon 1 Primers #5516, SimpleChIP® Human RPL30 Exon 3 Primers #7014, SimpleChIP® Human MyoD1 Exon 1 Primers #4490, and SimpleChIP® Human MYT-1 Exon 1 Primers #4493.

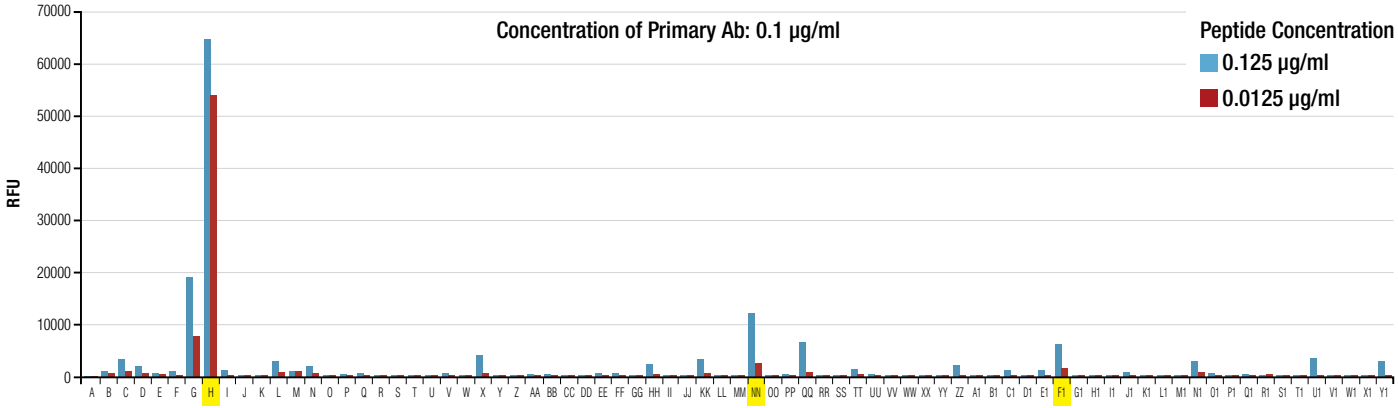
# Tri-Methyl-Histone H3 (Lys9) Antibody Performance Comparison

## Peptide Array

**Tri-Methyl-Histone H3 (Lys9) (D4W1U) Rabbit mAb #13969** is highly specific for tri-methyl-histone H3 (Lys9) and binding is not affected by methylation at Arg8 or phosphorylation at Thr6.



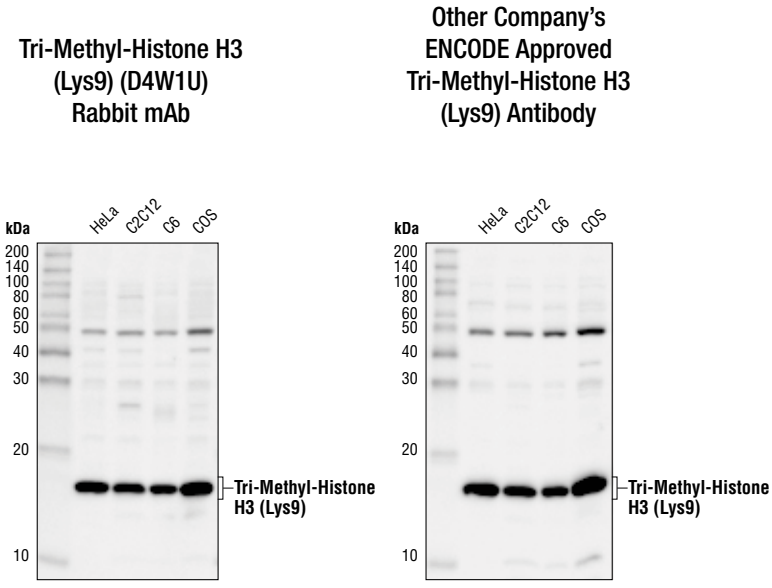

**Other Company's ENCODE Approved Tri-Methyl-Histone H3 (Lys9) Antibody** is highly specific for tri-methyl-histone H3 (Lys9). However, binding is reduced by methylation at Arg8 and phosphorylation at Thr6, indicating this antibody will not detect tri-methyl-histone H3 (Lys9) in the context of these multiple modifications in western blot, ChIP, and other assays.




### Peptide List

| | |
|---|---|
| A | H3 (Lys4) non-methyl |
| B | H3 (Lys4) mono-methyl |
| C | H3 (Lys4) di-methyl |
| D | H3 (Lys4) tri-methyl |
| E | H3 (Lys9) non-methyl |
| F | H3 (Lys9) mono-methy |
| G | H3 (Lys9) di-methyl |
| H | H3 (Lys9) tri-methyl |
| I | H3 (Lys27) non-methyl |
| J | H3 (Lys27) mono-methyl |
| K | H3 (Lys27) di-methyl |
| L | H3 (Lys27) tri-methyl |
| M | H3 (Lys36) non-methyl |
| N | H3 (Lys36) mono-methyl |
| O | H3 (Lys36) di-methyl |
| P | H3 (Lys36) tri-methyl |
| Q | H3 (Lys79) non-methyl |
| R | H3 (Lys79) mono-methyl |
| S | H3 (Lys79) di-methyl |
| T | H3 (Lys79) tri-methyl |
| U | H4 (Lys20) non-methyl |
| V | H4 (Lys20) mono-methyl |
| W | H4 (Lys20) di-methyl |
| X | H4 (Lys20) tri-methyl |
| Y | H2A (Lys5) non-methyl |
| Z | H2A (Lys5) mono-methyl |
| AA | H2A (Lys5) di-methyl |
| BB | H2A (Lys5) tri-methyl |
| CC | H3 (Thr3) phospho/(Lys4) mono-methyl |
| DD | H3 (Thr3) phospho/(Lys4) di-methyl |
| EE | H3 (Thr3) phospho/(Lys4) tri-methyl |
| FF | H3 (Arg2) symmeric-di-methyl/(Lys4) mono-methyl |
| GG | H3 (Arg2) symmeric-di-methyl/(Lys4) di-methyl |
| HH | H3 (Arg2) symmeric-di-methyl/(Lys4) tri-methyl |
| II | H3 (Arg2) asymmeric-di-methyl/(Lys4) mono-methyl |
| JJ | H3 (Arg2) asymmeric-di-methyl/(Lys4) di-methyl |
| KK | H3 (Arg2) asymmeric-di-methyl/(Lys4) tri-methyl |
| LL | H3 (Arg8) symmetric-di-methyl/(Lys9) mono-methyl |
| MM | H3 (Arg8) symmetric-di-methyl/(Lys9) di-methyl |
| NN | H3 (Arg8) symmetric-di-methyl/(Lys9) tri-methyl |
| OO | H3 (Lys9) mono-methyl/(Ser10) phospho |
| PP | H3 (Lys9) di-methyl/(Ser10) phospho |
| QQ | H3 (Lys9) tri-methyl/(Ser10) phospho |
| RR | H3 (Arg26) asymmetric-di-methyl/(Lys27) mono-methyl |
| SS | H3 (Arg26) asymmetric-di-methyl/(Lys27) di-methyl |
| TT | H3 (Arg26) asymmetric-di-methyl/(Lys27) tri-methyl |
| UU | H3 (Lys27) mono-methyl/(Ser28) phospho |
| VV | H3 (Lys27) di-methyl/(Ser28) phospho |
| WW | H3 (Lys27) tri-methyl/(Ser28) phospho |
| XX | H3 (Lys9) mono-methyl/(Ser10/Thr11) phospho |
| YY | H3 (Lys9) di-methyl/(Ser10/Thr11) phospho |
| ZZ | H3 (Lys9) tri-methyl/(Ser10/Thr11) phospho |
| A1 | H3 (Lys4) mono-methyl/(Thr6) phospho |
| B1 | H3 (Lys4) di-methyl/(Thr6) phospho |
| C1 | H3 (Lys4) tri-methyl/(Thr6) phospho |
| D1 | H3 (Thr6) phospho/(Lys9) mono-methyl |
| E1 | H3 (Thr6) phospho/(Lys9) di-methyl |
| F1 | H3 (Thr6) phospho/(Lys9) tri-methyl |
| G1 | H3 (Lys56) non-methyl |
| H1 | H3 (Lys56) mono-methyl |
| I1 | H3 (Lys56) di-methyl |
| J1 | H3 (Lys56) tri-methyl |
| K1 | H1.4 (Lys26) |
| L1 | H1.4 (Lys26) mono-methyl |
| M1 | H1.4 (Lys26) di-methyl |
| N1 | H1.4 (Lys26) tri-methyl |
| O1 | H1.4 (Lys26) mono-methyl/(Ser27) phospho |
| P1 | H1.4 (Lys26) di-methyl/(Ser27) phospho |
| Q1 | H1.4 (Lys26) tri-methyl/(Ser27) phospho |
| R1 | H2B (Lys5/Lys12/Lys15/Lys20) |
| S1 | H2B (Lys5) mono-methyl |
| T1 | H2B (Lys5) di-methyl |
| U1 | H2B (Lys5) tri-methyl |
| V1 | H4 (Lys5/Lys8/Lys12/Lys16) |
| W1 | H4 (Lys5) mono-methyl |
| X1 | H4 (Lys5) di-methyl |
| Y1 | H4 (Lys5) tri-methyl |

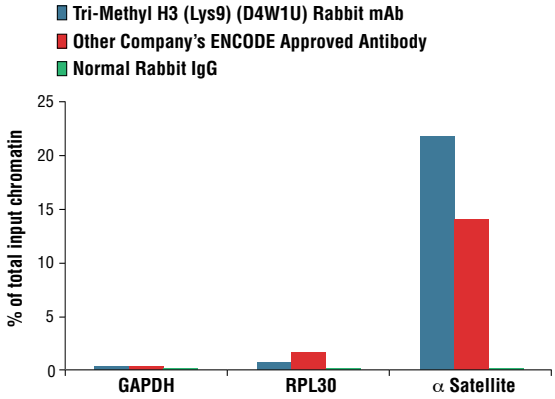
## Western Blot




Western blot analysis of cell extracts from various cell lines using Tri-Methyl-Histone H3 (Lys9) (D4W1U) Rabbit mAb **(left)** and the ENCODE approved antibody from the other company (used according to manufacturer's recommendation) **(right)**.

Although both antibodies are nearly indistinguishable by western blotting, the peptide array assay shows that binding of the other company's ENCODE approved antibody is blocked by known neighboring modifications with distinct biological functions. Therefore, the other company's antibody will not detect H3K9Me3 in the context of these multiple modifications in western blot, ChIP, and other assays.

## ChIP



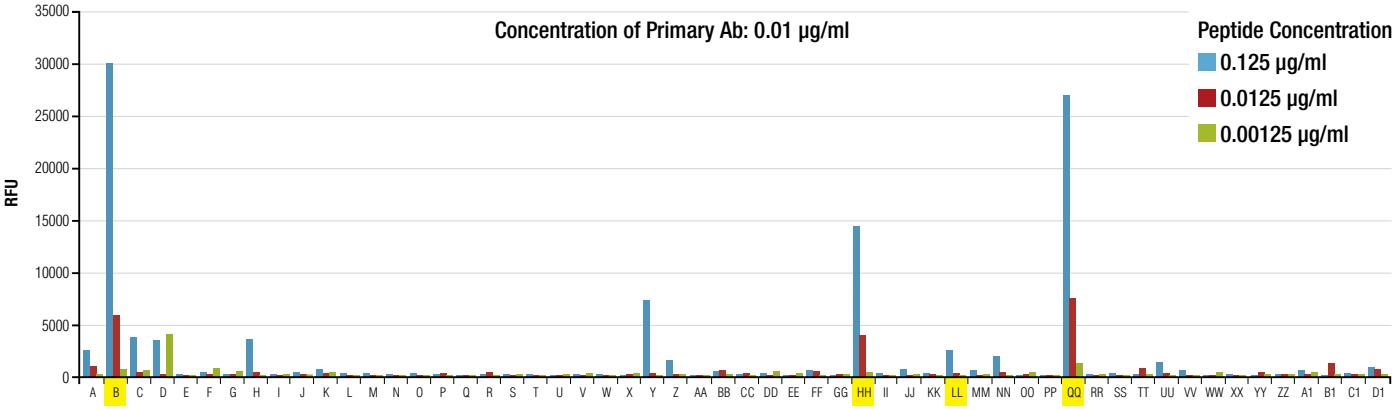

Chromatin immunoprecipitation was performed with cross-linked chromatin from 4 x $10^6$ HeLa cells and either 10 µl of Tri-Methyl H3 (Lys9) (D4W1U) Rabbit mAb, 4 µl of Tri-Methyl H3 (Lys9) ENCODE approved antibody from the other company (used according to manufacturer's recommendation), or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. The enriched DNA was quantified by real-time PCR using primers to the indicated loci.

Tri-methylation at Histone H3 on Lys9 is associated with inactive regions of the genome. As expected, Tri-Methyl-Histone H3 (Lys9) (D4W1U) Rabbit mAb effectively enriches the inactive α Satellite region and does not enrich active genes like GAPDH and RPL30. The ENCODE approved antibody from the other company provided significantly less target enrichment.

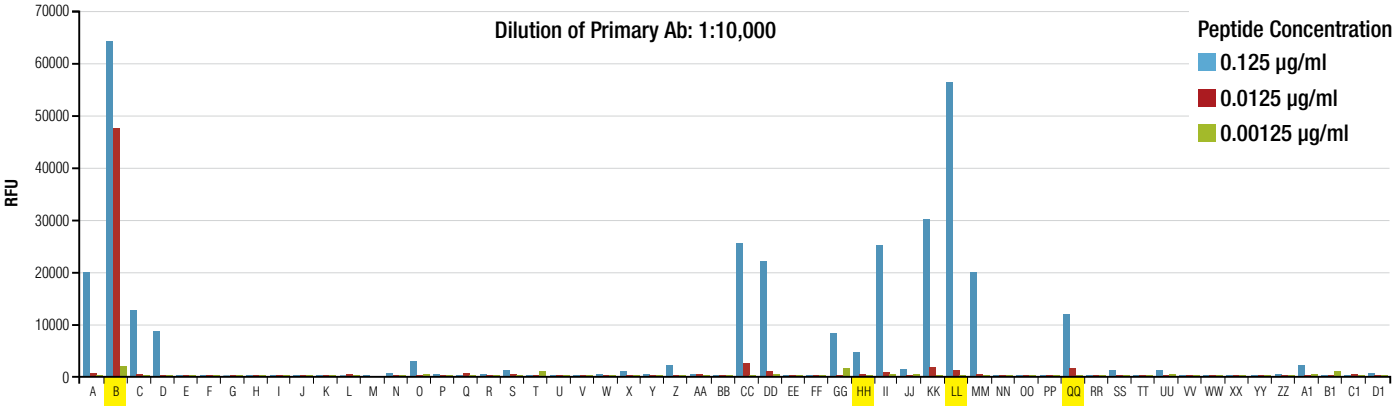
# Acetyl-Histone H3 (Lys9) Antibody Performance Comparison

## Peptide Array

**Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb #9649** is specific for acetyl-histone H3 (Lys9). It shows minimal cross-reactivity with other acetyl-lysine residues. Its binding is reduced by phosphorylation at Ser10 but is not affected by methylation at Arg8 or phosphorylation at Thr6.




**Other Company's ENCODE Approved Acetyl-Histone H3 (Lys9) Antibody** binding is not reduced by phosphorylation at Ser10, but it cross-reacts with other acetyl-lysine residues and its binding is affected by methylation at Arg8 and phosphorylation at Thr6. This data suggests the antibody will cross-react with other acetyl-lysines in additional assays, such as western blot, ChIP, etc.



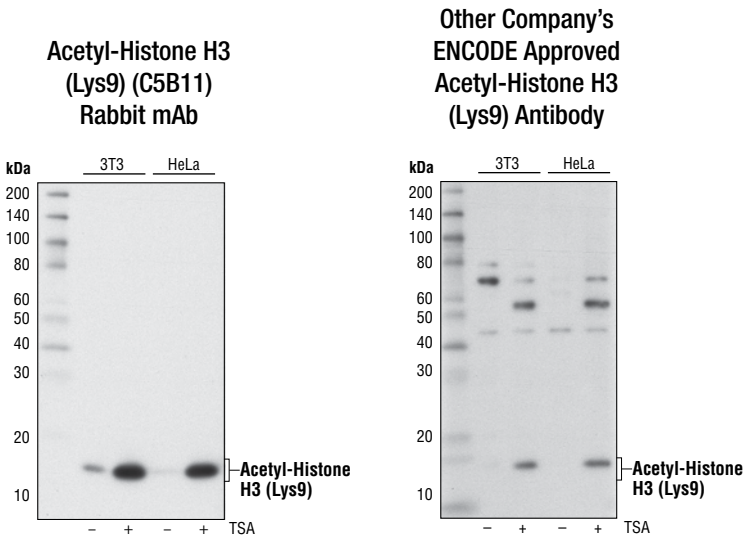

### Peptide List

| | |
|---|---|
| **A** | H3 (Lys9/Lys14/Lys18) |
| **B** | H3 (Lys9) acetyl |
| **C** | H3 (Lys14) acetyl |
| **D** | H3 (Lys18) acetyl |
| **E** | H3 (Lys23) |
| **F** | H3 (Lys23) acetyl |
| **G** | H3 (Lys27) |
| **H** | H3 (Lys27) acetyl |
| **I** | H3 (K36) |
| **J** | H3 (K36) acetyl |
| **K** | H3 (Lys56) |
| **L** | H3 (Lys56) acetyl |
| **M** | H4 (Lys5/Lys8/Lys12/Lys16) |
| **N** | H4 (Lys5) acetyl |
| **O** | H4 (Lys8) acetyl |
| **P** | H4 (Lys12) acetyl |
| **Q** | H4 (Lys16) acetyl |
| **R** | H4 (Lys20) |
| **S** | H4 (Lys20) acetyl |

| | |
|---|---|
| **T** | H4 (Lys91) |
| **U** | H4 (Lys91) acetyl |
| **V** | H2A |
| **W** | H2A (Lys5) acetyl |
| **X** | H2B (Lys5/Lys12/Lys15/Lys20) |
| **Y** | H2B (Lys5) acetyl |
| **Z** | H2B (Lys12) acetyl |
| **AA** | H2B (Lys15) acetyl |
| **BB** | H2B (Lys20) acetyl |
| **CC** | H3 (Lys4) |
| **DD** | H3 (Lys4) acetyl |
| **EE** | H3 (Lys79) |
| **FF** | H3 (Lys79) acetyl |
| **GG** | H3 (Thr3) phospho/(Lys4) acetyl |
| **HH** | H3 (Arg8) symmetric-di-methyl/(Lys9) acetyl |
| **I I** | H3 (Arg2) asymmetric-di-methyl/(Lys4) acetyl |
| **JJ** | H3 (Arg17) asymmetric-di-methyl/(Lys18) acetyl |
| **KK** | H3 (Arg2) symmetric-di-methyl/(Lys4) acetyl |
| **LL** | H3 (Lys9) acetyl/(Ser10) phospho |

| | |
|---|---|
| **MM** | H3 (Lys9) acetyl/(Ser10/Thr11) phospho |
| **NN** | H3 (Arg26) asymmetric-di-methyl/(Lys27) acetyl |
| **OO** | H3 (Lys27) acetyl/(Ser28) phospho |
| **PP** | H3 (Lys4) acetyl/(Thr6) phospho |
| **QQ** | H3 (Thr6) phospho/(Lys9) acetyl |
| **RR** | H4 (Arg3) asymmetric-di-methyl/(Lys5) acetyl |
| **SS** | H4 (Arg3) symmetric-di-methyl/(Lys5) acetyl |
| **TT** | H1.4 (Lys26) |
| **UU** | H1.4 (Lys26) acetyl |
| **VV** | H1.4 (Lys26) acetyl/(Ser27) phospho |
| **WW** | H2AX (Lys5) |
| **XX** | H2AX (Lys5) acetyl |
| **YY** | H2AZ (Lys4/Lys7/Lys11/Lys13/Lys15) |
| **ZZ** | H2AZ (Lys4) acetyl |
| **A1** | H2AZ (Lys7) acetyl |
| **B1** | H2AZ (Lys11) acetyl |
| **C1** | H2AZ (Lys13) acetyl |
| **D1** | H2AZ (Lys15) acetyl |

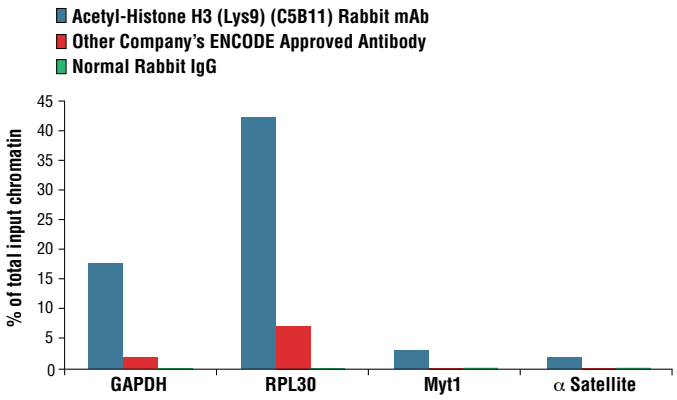
## Western Blot




Western blot analysis of extracts from 3T3 and HeLa cells, untreated (-) or treated with TSA (+), using Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb or the ENCODE approved antibody from the other company (used according to the manufacturer's recommendations).

The Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb detects a single band at the appropriate molecular weight. The ENCODE approved antibody from the other company is weaker than the CST antibody and also cross-reacts with numerous unidentified proteins.

## ChIP

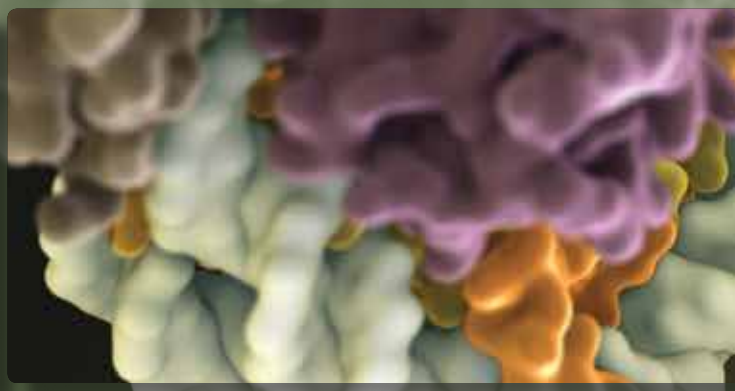



Chromatin immunoprecipitation was performed with cross-linked chromatin from 4 x $10^6$ cells. HeLa cells and either 10 µl of Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb, 10 µl of Acetyl-H3 (Lys9) ENCODE approved antibody from the other company (used according to manufacturer's recommendation), or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. The enriched DNA was quantified by real-time PCR using primers to the indicated loci.

Acetylation at Histone H3 on Lys9 is associated with actively transcribed genes such as GAPDH and RPL30. As expected, Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb effectively enriches for these regions of the genome and does not enrich for inactive genes like Myt1 and α Satellite. The ENCODE approved antibody from the other company showed significantly less enrichment of the target loci.

Cell Signaling TECHNOLOGY®

Cell Signaling Technology (CST) is a private, family-owned company, founded by scientists and dedicated to providing high quality research tools to the biomedical research community. Our 400 employees operate worldwide from our headquarters in Danvers, Massachusetts, and our offices in the Netherlands, China, and Japan. As scientists ourselves, we believe an antibody is only as good as the research it enables. For this reason, we are actively engaged in the development of technologies to facilitate signaling analysis and mechanistic cell biology research. And, the same scientists who produce and validate our primary antibodies are available to provide technical support for customers. In this way, we are able to supply customers with both the reagents and the information they need to achieve consistent, reliable results at the research bench.

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