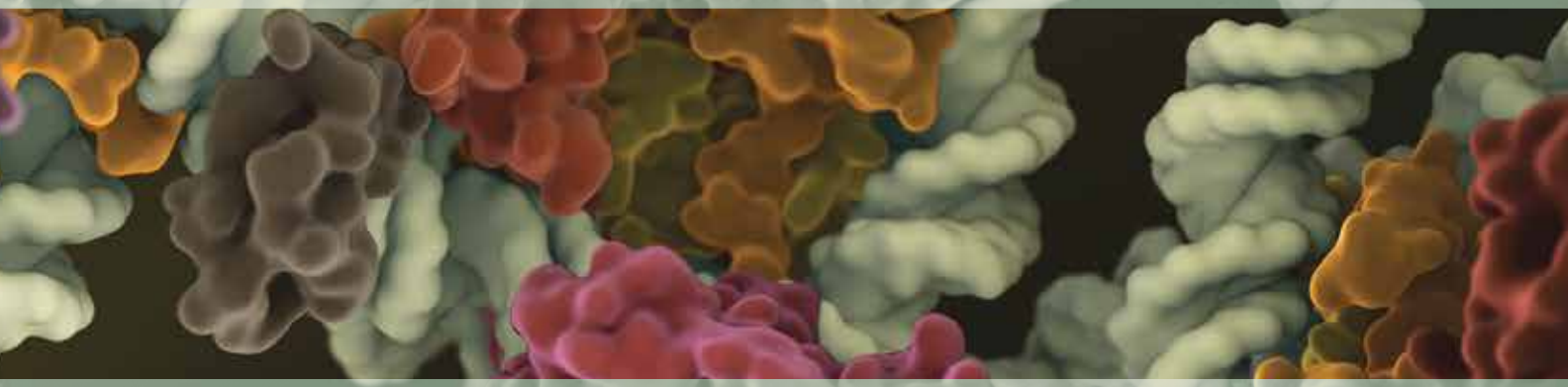

ANTIBODY PERFORMANCE COMPARISON

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

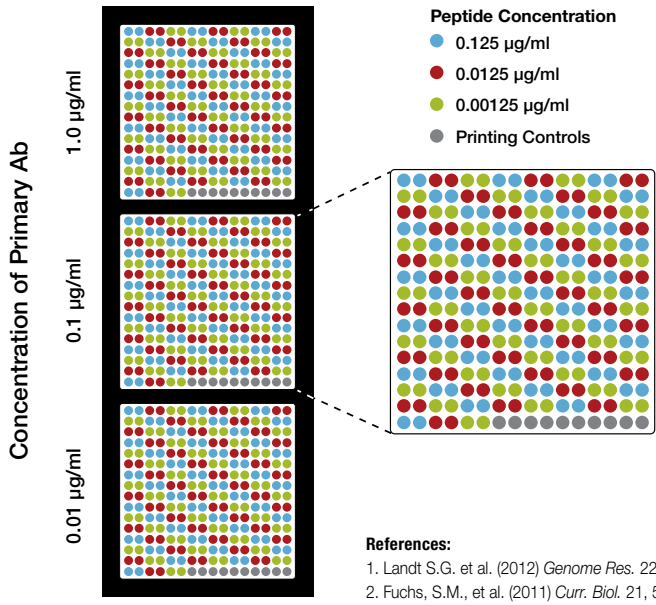
Peptides with mono-,di-, tri-methyl, acetyl-, or unmodified lysine are spotted onto nitrocellulose either alone or in combination with a known neighboring histone modification (e.g., histone H3K4Me3 and H3T3Phos), as indicated in the diagram. A similar array is used for testing methyl-arginine antibodies.

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.



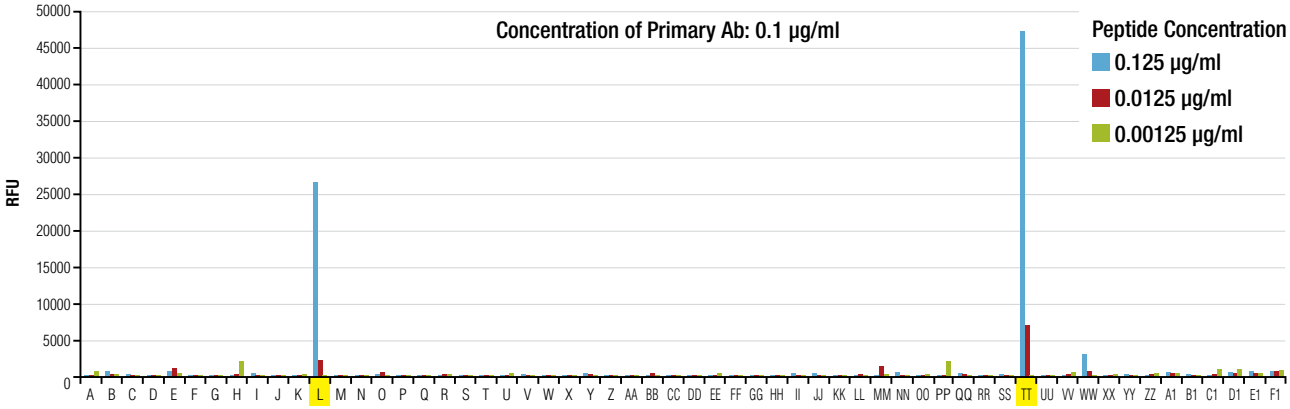
References:

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

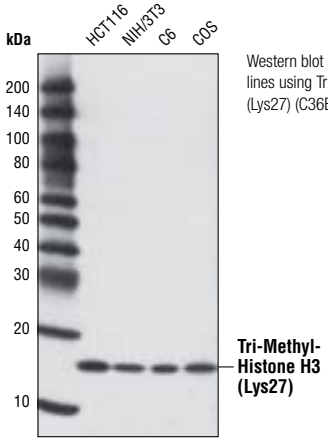
Tri-Methyl Histone H3 (Lys27) (C36B11) Rabbit mAb #9733 is highly specific for tri-methyl-histone H3 (Lys27) and is not affected by methylation at Arg26.



Peptide List

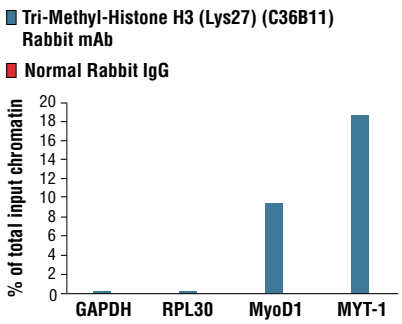
A	H3 (Lys4) non-methyl
B	H3 (Lys4) mono-methyl
C	H3 (Lys4) di-methyl
D	H3 (Lys4) tri-methyl
E	H3 (Lys9) non-methyl
F	H3 (Lys9) mono-methyl
G	H3 (Lys9) di-methyl
H	H3 (Lys9) tri-methyl
I	H3 (Lys27) non-methyl
J	H3 (Lys27) mono-methyl
K	H3 (Lys27) di-methyl
L	H3 (Lys27) tri-methyl
M	H3 (Lys36) non-methyl
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

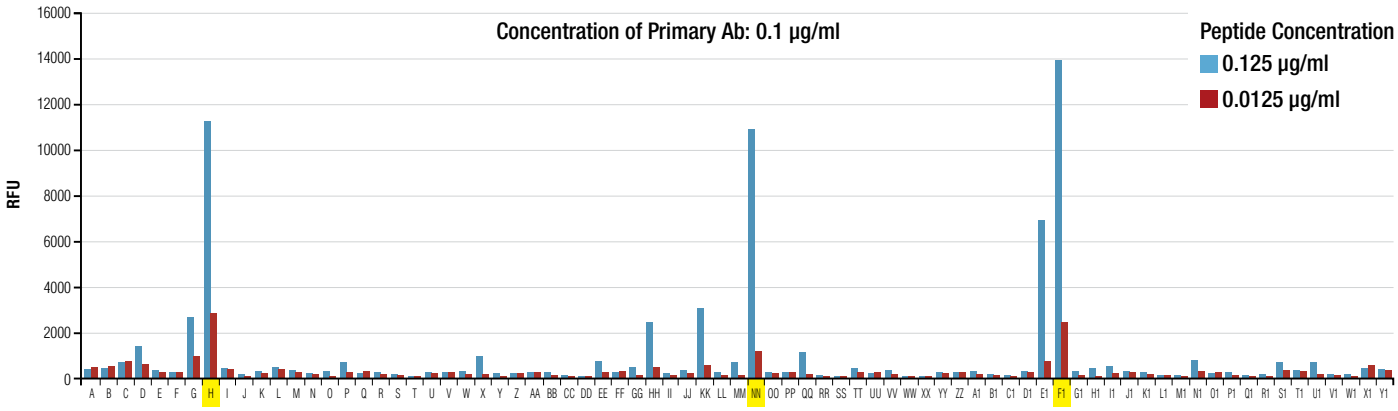


Chromatin immunoprecipitation was performed with crosslinked chromatin from 4 x 10^6 HeLa cells and either 10 µl of Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb, or 2 µl of Normal Rabbit IgG #2729, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified 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.

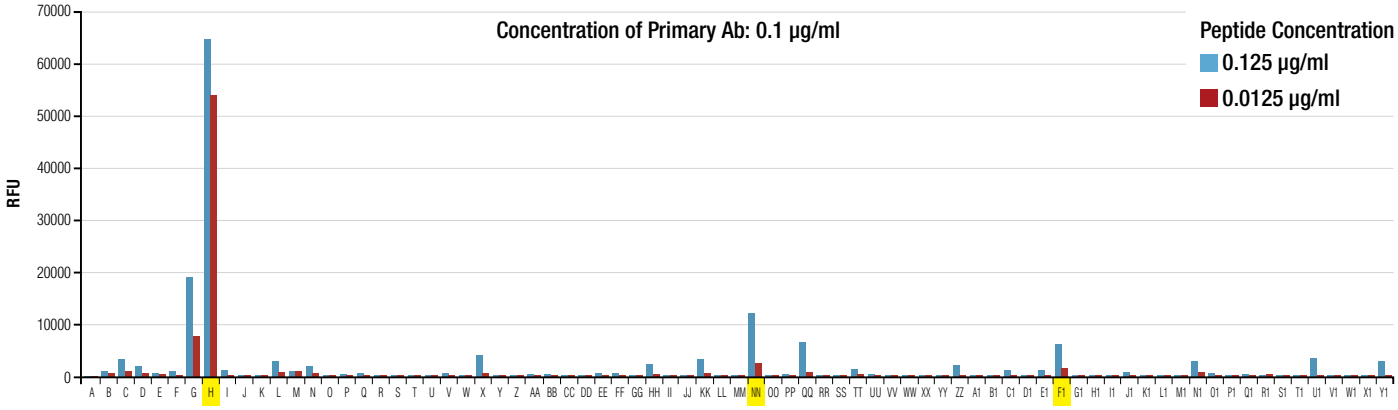
Tri-Methyl-Histone H3 (Lys9) Antibody Performance Comparison

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.



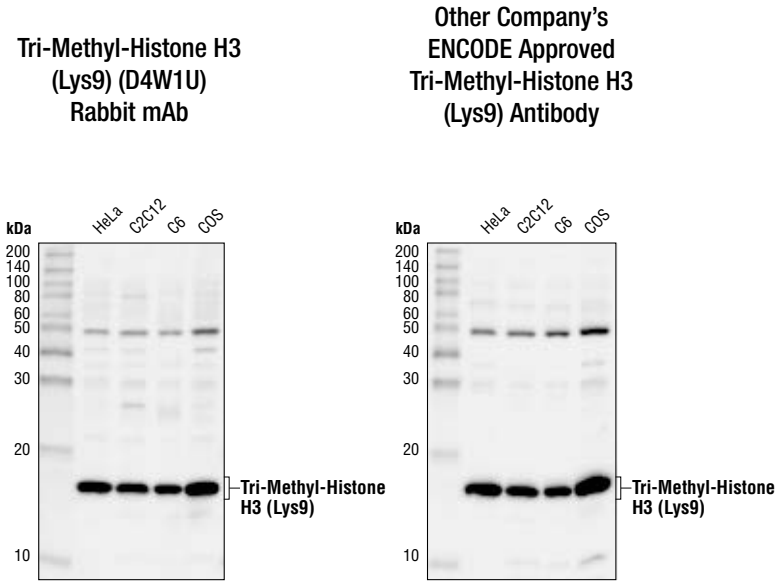
Other Company's ENCODE Approved Tri-Methyl-Histone H3 (Lys9) Antibody is highly specific for tri-methyl-histone H3 (Lys9). However, binding is reduced by methylation at Arg8 and phosphorylation at Thr6, indicating this antibody will not detect tri-methyl-histone H3 (Lys9) in the context of these multiple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

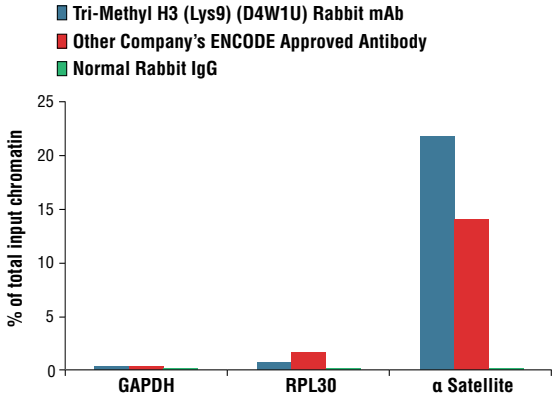
Western Blot



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



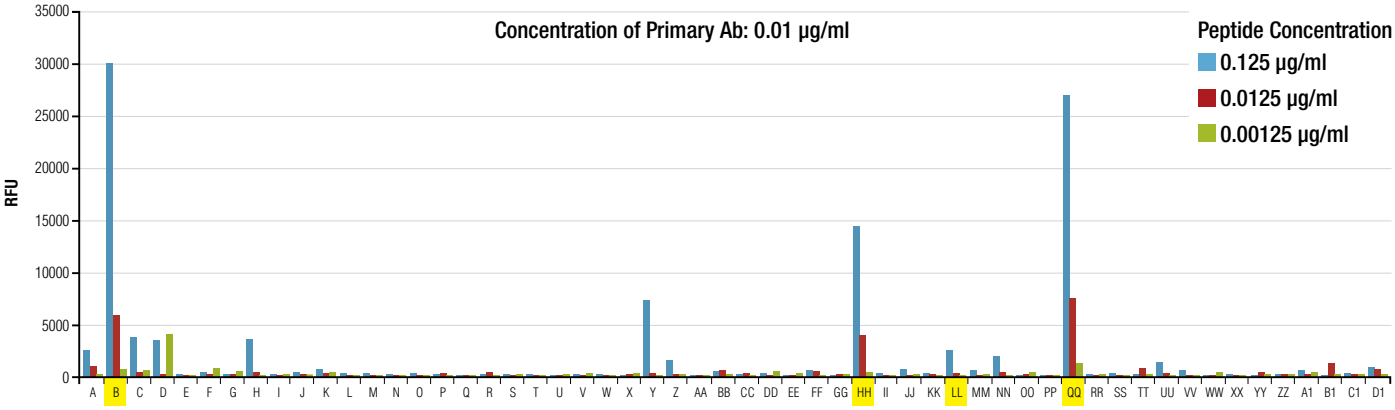
Chromatin immunoprecipitation was performed with cross-linked chromatin from 4 x 10^6 HeLa cells and either 10 µl of Tri-Methyl H3 (Lys9) (D4W1U) Rabbit mAb, 4 µl of Tri-Methyl H3 (Lys9) ENCODE approved antibody from the other company (used according to manufacturer's recommendation), or 2 µl of Normal 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.

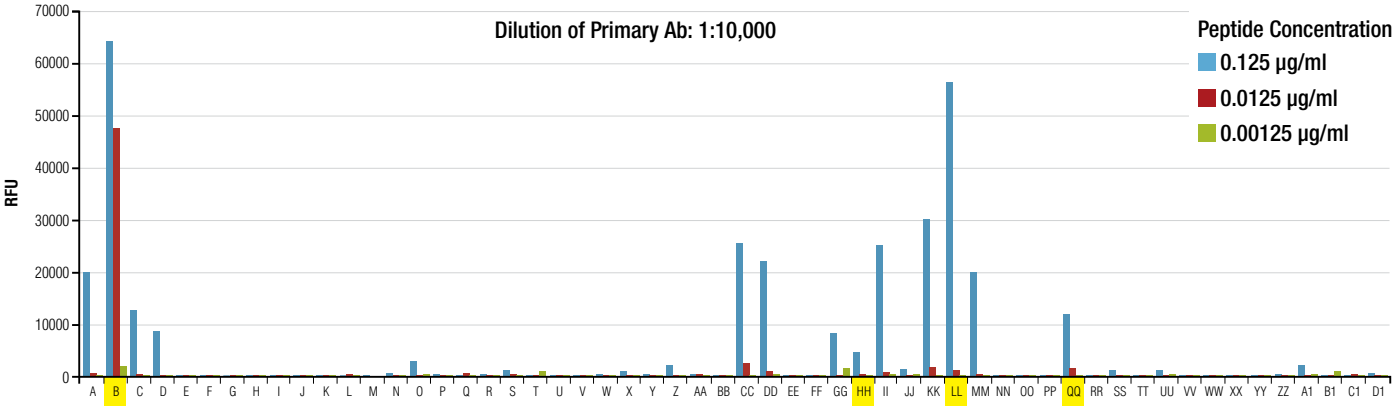
Acetyl-Histone H3 (Lys9) Antibody Performance Comparison

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.



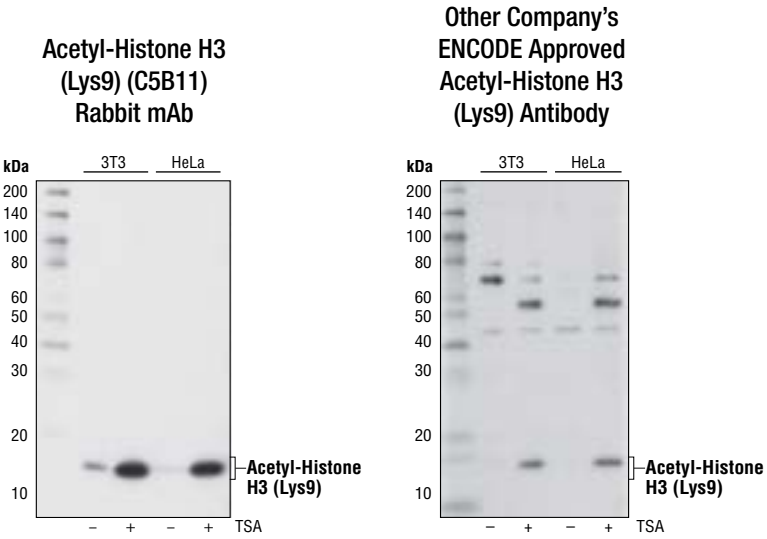
Peptide List

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
II	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

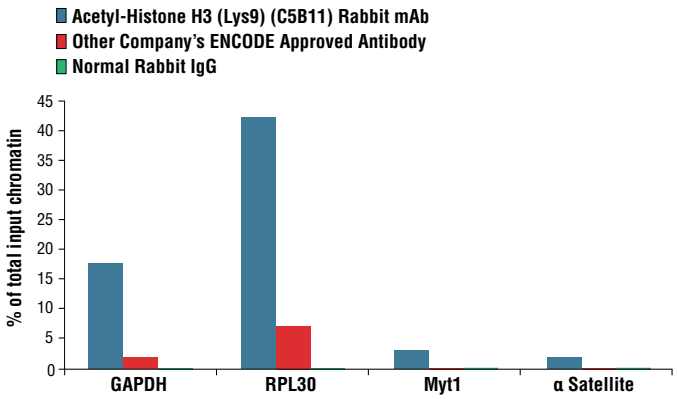
Western Blot



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

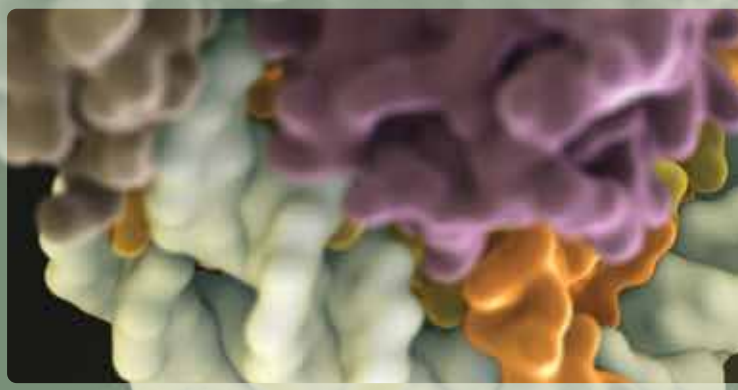


Chromatin immunoprecipitation was performed with cross-linked chromatin from 4 x 10^6 cells. HeLa cells and either 10 µl of Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb, 10 µl of Acetyl-H3 (Lys9) ENCODE approved antibody from the other company (used according to manufacturer's recommendation), or 2 µl 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 *different* kind of life sciences company—one founded, owned, and run by active research scientists, with the highest standards of product and service quality, technological innovation, and scientific rigor for over 20 years. We consistently provide fellow scientists around the globe with best-in-class products and services to fuel their quests for discovery. CST is a company of caring people driven by a devotion to facilitating good science—a company committed to doing the right thing for our Customers, our communities, and our planet.



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