Anti-dimethyl-Histone H3 (Lys4)

Polyclonal Antibody

Cat. # 07-030

Lot # 2477948

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

pack size: 200 µL

Store at -20ºC

Certificate of Analysis

Applications	Species Cross Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, ChIP, IC, DB, ChIP-Seq, PIA	H, M, T, Dr, Ce	N/A	N-Terminus	Rb	~17 kDa	NP_003484

Background

Histone H3 is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N-terminal tail, H3 is involved with the structure of the nucleosomes of the 'beads on a string' structure. The N-terminal tail of histone H3 protrudes from the globular nucleosome core and can undergo several different types of epigenetic modifications that influence cellular processes. These modifications include the covalent attachment of methyl or acetyl groups to lysine and arginine amino acids and the phosphorylation of serine or threonine.

Presentation

Rabbit polyclonal antiserum containing 0.05% sodium azide.

Specificity

Recognizes the N-terminus of Histone H3 dimethylated at Lys4, MW ~17 kDa. Specificity demonstrated by Dot Blot using unmodified and various modified peptides.

Species Cross-reactivity

Human, Mouse, tetrahymena, Drosophila, and C. elegans. Broad species cross-reactivity is expected.

Immunogen

KLH conjugated linear peptide corresponding to human Histone H3 dimethylated at Lys4.

Molecular Weight

~17 kDa. An uncharacterized band at ~37 kDa may be observed in some cell lysates.

Method of Purification

Unpurified

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

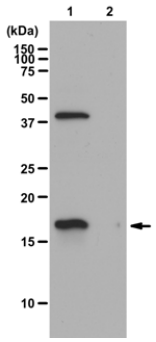
Control

Recombinant Histone H3

Quality Control Testing

Evaluated by Western Blot on HeLa acid extract.

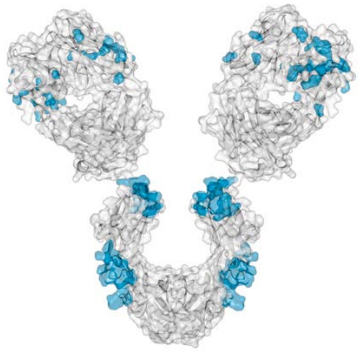
Western Blot Analysis: 1:500 dilution of this antibody detected dimethyl Histone H3 (Lys4) in acid extracted proteins from HeLa cells.



Western Blot Analysis:
Representative lot data.

HeLa acid extract (lane 1) and recombinant Histone H3 (lane 2) were probed with a 1:500 dilution of anti-dimethyl Histone H3 (Lys4). Proteins were visualized using a Donkey Anti-Rabbit IgG secondary antibody conjugated to HRP a chemiluminescence detection system.

Arrow indicates dimethyl-Histone H3 (Lys4) (~17 kDa). An uncharacterized band at ~37 kDa may be observed in some cell lysates.



References

1. Chen, D., *et al.* (1999). *Science.* 284(5423):2174-2177.
2. Silva, J., *et al.* (2003). *Dev Cell.* 4(4):481-495.
3. Kohlmaier, A., *et al.* (2004). *PLoS Biol.* 2: E171.
4. Boggs, B., *et al.* (2002). *Nature Genetics.* 30: 73-76.
5. Strahl, B. D., *et al.* (1999). *Proc Natl Acad Sci.* USA. 96: 14967-14972.
6. Egelhofer, T. A., *et al.* (2011). *Nat Struct Mol Biol*. 18(1):91-93.

Additional Research Applications

ChIP-Seq Analysis: A representative lot of this antibody was used by three independent laboratories for ChIP-Seq (Sarah Elgin Lab, Washington University; Strome and Ahringer Lab, UC Santa Cruz/ University of Cambridge; Vincenzo Pirrotta Lab, Rutgers University). See Egelhofer, T.A., et al. (2011).Non-Lot Specific Tested Application 2:

APPLICATION LEGEND: **WB** Western Blotting **IP** Immunoprecipitation **IC** Immunocytochemistry **IF** Immunofluorescence **IH** Immunohistochemistry (Tissue) **LUMX** Luminex **ChIP** Chromatin Immunoprecipitation **DB** Dot Blotting **ChIP-Seq** Chromatin Immunoprecipitation-Sequence

SPECIES LEGEND: **H** Human **T** Tetrahymena **Rb** Rabbit **Dr** *Drosophila* **Ce** *C. elegans* **WR** Most Common Vertebrates **( )** Predicted Reactivity

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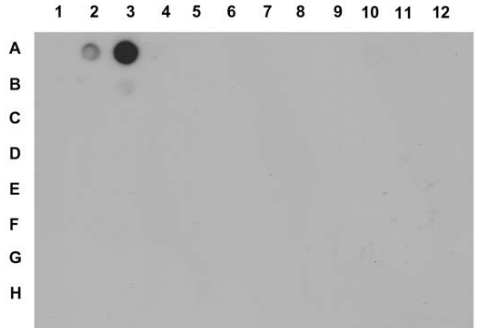
Additional Research Applications

Dot Blot Specificity Analysis: Unmodified and various modified Histone peptides (see Table 1) were probed with Anti-dimethyl-Histone H3 (Lys4) (1:500 dilution).



Dot Blot (Specificity) Analysis:
Representative lot data.

Unmodified and various modified Histone peptides (see Table 1) were probed with Anti-dimethyl-Histone H3 (Lys4) (1:500 dilution). Proteins were visualized using a Donkey Anti-Rabbit IgG secondary antibody conjugated to HRP and a chemiluminescence detection system.

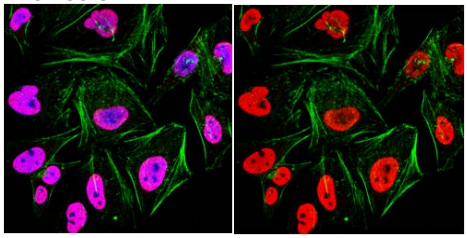
This antibody demonstrates slight cross-reactivity with monomethyl-Histone H3 (Lys4) at higher concentrations of peptide.

Table 1

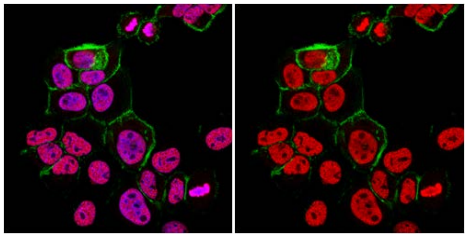
	1	2	3	4	5	6	7	8	9	10	11	12
A: 40 ng B: 4 ng	unmod. Histone H3 (Lys4)	monomethyl-Histone H3(Lys4)	dimethyl-Histone H3 (Lys4)	trimethyl-Histone H3 (Lys4)	unmod. Histone H3(Lys9)	monomethyl-Histone H3 (Lys9)	dimethyl-Histone H3 (Lys9)	trimethyl-Histone H3 (Lys9)	unmod. Histone H3 (Lys27)	monomethyl-histone H3 (Lys27)	dimethyl-Histone H3 (Lys27)	trimethyl-Histone H3 (Lys27)
C: 40 ng D: 4 ng	unmod. Histone H3 (Lys36)	monomethyl-Histone H3 (Lys36)	dimethyl-Histone H3 (Lys36)	trimethyl-Histone H3 (Lys36)	unmod. Histone H3 (Lys37)	monomethyl-Histone H3 (Lys37)	dimethyl-Histone H3 (Lys37)	trimethyl-Histone H3 (Lys37)	unmod. Histone H3 (Lys56)	monomethyl-Histone H3 (Lys56)	unmod. Histone H3 (Lys79)	monomethyl-Histone H3 (Lys79)
E: 40 ng F: 4 ng	dimethyl-Histone H3 (Lys79)	trimethyl-Histone H3 (Lys79)	unmod. Histone H4 (Lys20)	monomethyl-Histone H4 (Lys20)	dimethyl-Histone H4 (Lys20)	trimethyl-Histone H4 (Lys20)	unmod. Histone H1.0 (Lys26)	monomethyl-Histone H1.0 (Lys26)	dimethyl-Histone H1.0 (Lys26)	trimethyl-Histone H1.0 (Lys26)	unmod. Histone H2A (Lys127)	monomethyl-Histone H2A (Lys127)
G: 40 ng H: 4 ng	dimethyl-Histone H2A (Lys127)	trimethyl-Histone H2A (Lys127)	unmod. Histone H2A (Lys118/119)	monomethyl-Histone H2A (Lys118)	monomethyl-Histone H2A (Lys119)	Histone H2A (methyl-Lys118, ubiquityl-Lys119)	Histone H2A (ubiquityl-Lys118, methyl-Lys119)	unmod. Histone H2A (Lys17)	monomethyl-Histone H2A (Lys17)	dimethyl-Histone H2A (Lys17)	-	-

Immunocytochemistry Analysis: A 1:500 dilution from a representative lot detected dimetyl-Histone H3 (Lys4) in A431 and HeLa cells.

A431 Cells



HeLa Cells



Immunocytochemistry Analysis:
Representative lot data.

Confocal fluorescent analysis of A431 and HeLa cells using Anti-dimethyl-Histone H3 (Lys 4) (Red). Actin filaments have been labeled with Alexa Fluor® 488 dye-Phalloidin (Green). Nucleus is stained with DAPI (Blue). This antibody positively stains the nucleus.

Western Blot Analysis: A representative lot of this antibody was used by three independent laboratories for WB (Sarah Elgin Lab, Washington University; Strome and Ahringer Lab, UC Santa Cruz/ University of Cambridge; Vincenzo Pirrotta Lab, Rutgers University). See Egelhofer, T.A., et al. (2011).

Peptide Inhibition Analysis: A representative lot blocked dimethyl-Histone H3 (Lys27) in HeLa acid extract.

PROTOCOL

Western Blot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na_3VO_4; 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20-30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **1:500 dilution of anti-dimethyl-Histone H3 (Lys4)**, diluted in freshly prepared PBS-MLK overnight with agitation at 2-8°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

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Chromatin Immunoprecipitation Protocol

*Required Solutions

Protease Inhibitors:

1mM phenylmethylsulfonyl fluoride (PMSF), 1 µg/mL aprotinin and 1 µg/mL pepstatin A. We recommend using a PMSF stock solution less than one month old and add PMSF to the buffer just prior to use since PMSF has a half-life of about 30 minutes in aqueous solutions.

SDS Lysis Buffer (Catalog # 20-163): 1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.1.

ChIP Dilution Buffer (Catalog # 20-153): 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris, pH 8.1, 167 mM NaCl.

1. Cross-link histones to DNA by adding formaldehyde directly to culture medium to a final concentration of 1% and incubate for 10 minutes at 37°C. Seal culture vessels if returning the cultures to an incubator containing other cells.
2. Aspirate medium, wash and scrape cells with ice cold PBS containing protease inhibitors*.
3. Pellet cells for 4 minutes at 700 x g at 2-8°C.
4. Warm SDS Lysis Buffer* to room temperature to dissolve precipitated SDS and add protease inhibitors. Resuspend cell pellet in 200 µL SDS Lysis Buffer* for 10 minutes on ice.
5. Sonicate lysate to reduce DNA length to between 200 and 1000 basepairs. Cool samples on dry ice between pulses but do not freeze the samples. Remove debris by centrifugation for 10 minutes at 13,000 rpm at 2-8°C in a microcentrifuge.
6. Dilute supernatant fraction 10-fold in ChIP Dilution Buffer* with protease inhibitors added. Keep a portion of this chromatin solution (1%) to quantitate the amount of DNA present in different samples before immuno-precipitation.
7. To reduce nonspecific background, pre-clear the chromatin solution with 80 µL of Salmon Sperm DNA/Protein A Agarose (Catalog # 16-157) for 30 minutes at 2-8°C with agitation.
8. Pellet beads by a brief centrifugation and collect supernatant fraction.
9. Add 5 µL of anti-dimethyl Histone H3 to 1 mL of chromatin solution (supernatant fraction of step 7) and incubate overnight at 2-8°C with rotation. Save the other 1ml of chromatin solution for a no-antibody control.
10. Collect immune complexes with 60 µL of Salmon Sperm DNA/Protein A Agarose (Catalog # 16-157) for one hour at 2-8°C with rotation.
11. Prepare elution buffer (1%SDS, 0.1 M $NaHCO_3$).
12. Pellet beads by centrifugation and wash five times, for 3-5 minutes per wash, using the sequence of buffers listed below. Use 1 mL of each buffer per wash.
    a) Low Salt Immune Complex Wash Buffer (Catalog # 20-154): 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1. One wash.
    b) High Salt Immune Complex Wash Buffer (Catalog # 20-155): 500 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1. One wash.
    c) LiCl Immune Complex Wash Buffer (Catalog # 20-156): 0.25 M LiCl, 1% NP40, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1. One wash.
    d) TE Buffer (Catalog # 20-157): 0.10 mM Tris-HCl, 1 mM EDTA, pH 8.0. Two washes.
13. **Elute immune complexes by adding 250 µL elution buffer (see step 11) to the pelleted beads. Vortex briefly to mix and incubate at room temperature for 15 minutes with rotation. Spin down beads, carefully transfer the supernatant fraction (eluate) to another tube and repeat elution. Combine eluates.
14. Add 20 µL 5 M NaCl to the combined eluates and reverse crosslinks at 65°C for 4 hours.
15. Add 10 µL of 0.5 M EDTA, 20 µL 1 M Tris-HCl, pH 6.5, and 2 µL of 10 mg/mL Proteinase K to the eluate and incubate for one hour at 45°C.
16. Recover DNA by phenol/chloroform extraction and ethanol precipitation. Addition of an inert carrier, such as 20 µg glycogen or yeast RNA is

suggested. Wash pellets with 70% ethanol and slow to air day.

17. Resuspend pellets in an appropriate buffer or water. Detect specific sequences from no-antibody and immuno-precipitated samples by quantitative PCR or slot-blot. Include input and unbound DNA samples as controls. Conditions for PCR amplification must be determined empirically.

**Following washing of the beads, immunoprecipitated histone can be assessed by immunoblot analysis after boiling of the samples in Laemmli buffer for 10 minutes.

RELATED PRODUCTS

cat #	description
05-684	Anti-dimethyl-Histone H3 (Lys4), clone RR302
07-370	Anti-dimethyl (Lys4) dimethyl (Lys9) Histone H3
07-214	Anti-dimethyl-Histone H3 (Arg17)
04-768	Anti-dimethyl-Histone H3 (Lys9), clone MC554
04-835	Anti-dimethyl-Histone H3 (Lys79), clone NL59
07-521	Anti-dimethyl-Histone H3 (Lys9)
05-685	Anti-dimethyl-Histone H3 (Lys9), clone RR202
05-790	Anti-dimethyl-Histone H3 (Lys4), clone AW30
07-585	Anti-dimethyl-Histone H3 (Arg2)
07-421	Anti-dimethyl-Histone H3 (Lys27)
07-322	Anti-dimethyl-Histone H3 (Lys27)
17-648	ChIPAb+ Dimethyl-Histone H3 (Lys9)
04-808	Anti-dimethyl-Histone H3 (Arg2), clone 20.2
07-369	Anti-dimethyl-Histone H3 (Lys36)

RELATED PRODUCTS

cat #	description
WBAVDBASE	SNAP i.d.® Protein Detection System
WBAVDABTR	SNAP i.d. Antibody Collection Tray
WBAVDR0LL	SNAP i.d. Blot Roller
WBAVDBH03	SNAP i.d. Triple Well Blot Holder
WBAVDBH01	SNAP i.d. Single Well Blot Holder
WBAVDBH02	SNAP i.d. Double Well Blot Holder
IPVH00010	Immobilon®-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm membrane
IPFL00010	Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm membrane
IPVH07850	Immobilon-P 7 x 8.4 cm PVDF 0.45 mm membrane (sheet) 50/pk
ISEQ00010	Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm membrane
ISEQ07850	Immobilon-P 7 x 8.4 cm PVDF 0.2 mm membrane (sheet) 50/pk
IPFL07810	Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm membrane (sheet) 10/pk
WBKLS0100	Immobilon Western Chemilum HRP Substrate 100 mL
2060	Re-Blot™ Western Blot Recycling Kit
2500	Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	Blot Quick Blocker™ Membrane Blocking Agent 175G
WBLUC0500	Luminata Classico Western HRP substrate, 500 mL
WBLUR0500	Luminata Crescendo Western HRP substrate, 500 mL
AP182P	Donkey Anti-Rabbit IgG, HRP conjugate

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