Anti-acetyl-Histone H3 (Lys9)

Polyclonal Antibody

Cat. # 07-352

Lot # 2465196

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

pack size: 100 µL

Store at -20ºC

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, ChIP, BD, ChIP-Seq	H, WR, Y	IgG	a.a. 4-14	Rb	17 kDa	NM_003493

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

Antiserum containing 0.05% sodium azide and 30% glycerol.

Specificity

Recognizes acetyl-histone H3 (Lys9), Mr 17 kDa. An additional unknown protein was detected at Mr 23 kDa.

Species Cross-reactivity

Yeast and human. Broad species cross-reactivity is expected.

Immunogen

Ovalbumin-conjugated, synthetic peptide (KQTAR$_{Ac}$KSTGGK-C) corresponding to amino acids 4-14 of yeast histone H3 acetylated on lysine 9, with a C-terminal cysteine added for conjugation purposes.

Molecular Weight

17 kDa

Storage and Handling

Stable for 1 year at -20°C from date of receipt. For maximum recovery of product, centrifuge the vial prior to removing the cap.

Control

TSA-treated HeLa and NIH/3T3 cells, osteosarcoma tissue.

Quality Control Testing

Routinely evaluated by western blot on acid extracts from sodium butyrate treated HeLa cells.

Western Blot Analysis: A 1:5000-1:10,000 dilution of this lot detected acetyl-Histone H3 (Lys9) in acid extracts from sodium butyrate treated HeLa cells (Catalog # 17-305), (Figure A).

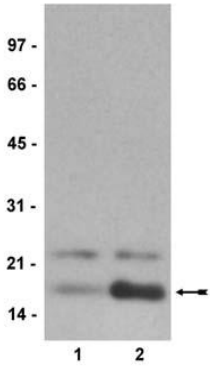
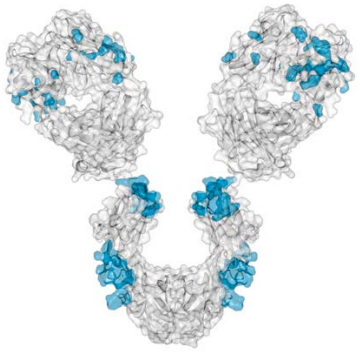


Figure A
Western Blot Analysis
Representative lot data.
Acid extracts from untreated (lane 1) and sodium butyrate treated (lane 2) HeLa cells (Catalog # 17-305) were resolved by electrophoresis, transferred to nitrocellulose and probed with antiacetyl-Histone H3 (Lys9), (1:5000 dilution).
Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.
Arrow indicates acetyl-Histone H3 (Lys9), (~17 kDa).

Additional Research Applications

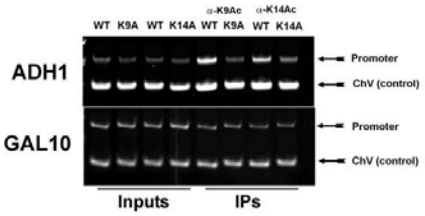
ChIP-Seq Analysis: A representative lot of this antibody was used by an independent laboratory for ChIP-Seq. See Wang, X. *et al*. (2009).



References

1. Benhamed, Moussa, *et al.* (2006). *Plant Cell.* 18: 2893-903.
2. Suka, N., *et al.* (2001). *Mol Cell.* 8: 473-9.
3. Wang, X. *et al*. (2009). *The Plant Cell.* Vol. 21: 1053–1069.

Figure B Chromatin Immunoprecipitation
Representative lot data.



Chromatin Immunoprecipitation (ChIP): An independent laboratory has shown this antibody preferentially immunoprecipitates chromatin from wild type yeast and not from a yeast strain containing a Lysine substitution to Alanine at residue 9 (Figure B).

Additional Research Applications

Beadlyte ® Histone-Peptide Specificity Assay: A 1:1000-1:5000 dilution of this lot was incubated with histone H3 peptides containing various modifications conjugated to Luminex® microspheres. (Figure C, page two). No cross-reactivity with peptides containing acetyl-lysine 14 or acetyl-lysine 27 was observed.

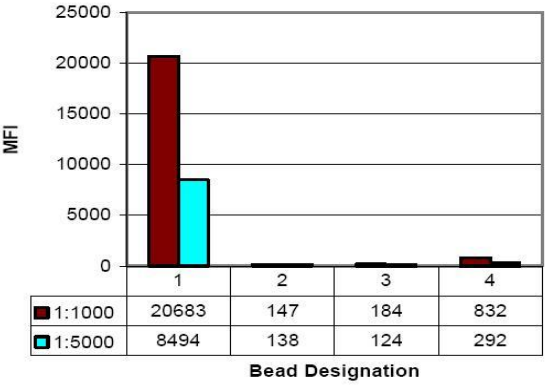


Figure C
Beadlyte® Histone-Peptide Specificity Assay
Representative data from a previous lot.
1:1000-1:5000 dilutions were incubated with a cocktail of microspheres conjugated to Histone H3 peptides with the following modifications:

1. acetyl-lysine 9
2. acetyl-lysine 14
3. acetyl-lysine 27
4. no modifications

Unbound antibody was then removed by filtration. Peptideantibody complexes were incubated with a biotin-conjugated anti-rabbit secondary antibody followed by incubation with a phycoerythrin-streptavidin conjugate. Fluorescence was read on a Luminex® 100™ instrument. Median Fluorescence Intensity (MFI) is plotted.

PROTOCOL

Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on an acid-extracted protein sample (see protocol below) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200) in TBS-MLK) for 1.5 hours at room temperature with constant agitation.
3. Incubate the nitrocellulose with a **1:5000-1:10,000 dilution of anti-acetyl-Histone H3 (Lys9)**, diluted in freshly prepared TBS-MLK for 1.5 to 2 hours at room temperature with constant agitation or overnight with agitation at 4°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 TBS-MLK for 30 minutes with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 10 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Acid Extraction of Proteins from HeLa Cells

1. Grow cells to 70% confluency in DMEM supplemented with 10% FBS.
2. Add sodium butyrate (100 mM sterile stock solution), which inhibits histone deacetylases, to a final concentration of 5 mM and continue to grow the cells for 24 hours.
3. Scrape the cells from the plate.
4. Pellet the cells by centrifugation at 200 x g for 10 minutes.
5. Decant the supernatant fraction.
6. Suspend the cells with 10-15 volumes of PBS and centrifuge at 200 x g for 10 minutes.
7. Decant supernatant fraction (PBS wash).
8. Suspend the cell pellet in 5-10 volumes of **lysis buffer.**
9. Add hydrochloric acid to a final concentration of 0.2 M (0.2 N). **Use polypropylene tubes.**
10. Incubate on ice for 30 minutes.
11. Centrifuge at 11,000 x g for 10 minutes at 4°C.
12. Keep the supernatant fraction, which contains the acid soluble proteins, and discard the acid-insoluble pellet.
13. Dialyze the supernatant against 200 mL 0.1 M (0.1 N) acetic acid, twice for 1-2 hours each.
14. Dialyze three times against 200 mL H20 for 1 hour, 3 hours, and overnight, respectively. The protein can be quantified and lyophilized or stored at -70°C.

Lysis buffer:

10 mM HEPES, pH 7.9 *0.5 mM DTT
1.5 mM MgCl2 *1.5 mM PMSF
10 mM KCl

***Add PMSF and DTT just prior to use of the buffer.**

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RELATED PRODUCTS (specific)

cat #	description
07-539	Anti-acetyl-Histone H3 (Lys 4)
07-353	Anti-acetyl-Histone H3 (Lys14)
07-354	Anti-acetyl-Histone H3 (Lys18)
07-355	Anti-acetyl-Histone H3 (Lys23)
07-360	Anti-acetyl-Histone H3 (Lys27)
07-750	Anti-acetyl-Histone H3 (Lys79)
07-593	Anti-acetyl-Histone H3 (Lys9/18)
07-370	Anti-dimethyl (Lys4) dimethyl (Lys9) Histone H3
07-608	Anti-dimethyl Histone H3 (Lys23)
07-214	Anti-dimethyl-Histone H3 (Arg17)
05-808	Anti-dimethyl-Histone H3 (Arg2)
07-215	Anti-dimethyl-Histone H3 (Arg26)
07-427	Anti-dimethyl-Histone H3 (Lys14)
07-452	Anti-dimethyl-Histone H3 (Lys27)
07-369	Anti-dimethyl-Histone H3 (Lys36)
07-652	Anti-dimethyl-Histone H3 (Lys37)
07-030	Anti-dimethyl-Histone H3 (Lys4)
05-835	Anti-dimethyl-Histone H3 (Lys79), clone NL59
07-212	Anti-dimethyl-Histone H3 (Lys9)
06-755	Anti-Histone H3
07-448	Anti-monomethyl-Histone H3 (Lys27)
07-548	Anti-monomethyl-Histone H3 (Lys36)
07-395	Anti-monomethyl-Histone H3 (Lys9)
05-713	Anti-monomethyl-Histone H3 (Lys9), clone RR103
05-817	Anti-phospho-Histone H3 (Ser10), clone MC463
06-570	Anti-phospho-Histone H3 (Ser10), Mitosis Marker
07-145	Anti-phospho-Histone H3 (Ser28)
05-789	Anti-phospho-Histone H3 (Thr11), clone MC83
05-809	Anti-trimethyl (Lys9)-phospho (Ser10)-Histone H3
05-801	Anti-trimethyl-Histone H3 (Lys36), clone MC86
07-473	Anti-trimethyl-Histone H3 (Lys4)
17-622	ChIPAb+ Trimethyl-Histone H3 (Lys27)
17-625	ChIPAb+ Trimethyl-Histone H3 (Lys9)
12-568	Trimethyl-Histone H3 (Lys9) Peptide, biotin conjugate
17-245	Acetyl-Histone H3 Immunoprecipitation (ChIP) Assay Kit
12-348	Goat Anti-Rabbit IgG
UFC7 PCR 50	Montage PCR
UFC7 PC2 50	Montage PCR

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPFL00010	Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPVH07850	Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 um
ISEQ07850	Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0050	IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	Spray & Glow™ ECL Western Blotting 40 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
2170	CHEMIBLOCKER-1LT
20-200	IMMUNOBLOT BLOCKING REAGENT 20G
12-302	EGF-Stimulated A431 Cell Lysate
12-349	Goat Anti-Mouse IgG, HRP conjugate
12-110	Phosphotyrosine control (EGF-stim A431 cell lysate)

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