Anti-trimethyl-Histone H3 (Lys9)

Polyclonal Antibody

Cat. # 07-442

Lot # 2475742

pack size: 100 µg

Store at -20ºC

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

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, PIA, BD, DB, IC, ChIP-Seq	Ch, H, M, R, Ce	IgG	N/A	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

Purified rabbit polyclonal IgG in buffer containing 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide and 30% glycerol.

Concentration

1 mg/mL

Specificity

Trimethyl-histone H3 (Lys9). Broad species cross-reactivity expected.

Species Cross-reactivity

Human and *C. elegans*. Expected to cross-react with chicken, mouse and rat.

Immunogen

KLH-conjugated, 2X-branched synthetic peptide containing the sequence …AR[$_{me3}$K]ST… in which $_{me3}$K corresponds to trimethyl-lysine at residue 9 of human histone H3.

Molecular Weight

17 kDa

Method of Purification

Protein A chromatography

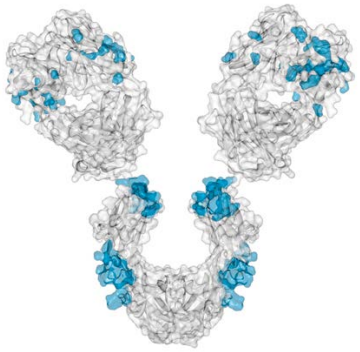
Storage and Handling

1 year at -20°C from date of shipment.

Handling Recommendations: Upon receipt, 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. Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

Control

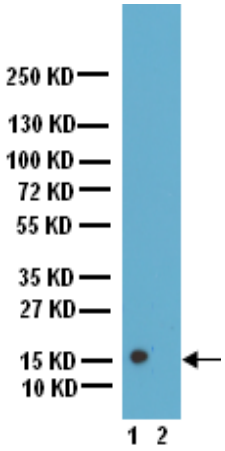
Acid-extracted proteins from HeLa cells.



Quality Control Testing

Routinely evaluated by Western Blot on HeLa acid extract and recombinant Histone H3 (cat. # 14-411).

Western Blot Analysis: 1:500 dilution of this lot detected trimethyl Histone H3 on 10 ug of HeLa acid extract but not on recombinant Histone H3.



Western Blot Analysis: Representative lot data. HeLa acid extract (lane 1) and recombinant Histone H3 (cat. # 14-411) (lane 2) were resolved by electrophoresis, transferred to PVDF membrane and probed with anti-trimethyl Histone H3 (1:500 dilution of this lot). Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein trimethyl Histone H3 (~17 kDa).

References

1. Kohlmaier, Alexander, *et al.* (2004). *PLoS Biol.* 2: E171.
2. Perez-Burgos, L., *et al.* (2004). *Methods Enzymol.* 376: 234-54.
3. Peters, A. H., *et al.* (2003). *Mol Cell.* 12: 1577-89.
4. Rea, S., *et al.* (2000). *Nature.* 406:593-9.
5. Ding, Yong, *et al.* (2007). *Plant Cell.* 19: 9-22.
6. Marban, Celine, *et al.* (2007). *EMBO J.* 26: 412-23.
7. Egelhofer, T.A., *et al*. (2011). *Nat Struct Mol Biol.* 18(1):91-93.

Additional Research Applications

A representative lot of this antibody was also used by an independent laboratory for WB (Strome and Ahringer Lab, UC Santa Cruz/ University of Cambridge). See Egelhofer, T.A., *et al*. (2011).

APPLICATION LEGEND: WB Western Blotting **DB** Dot Blot **IP** Immunoprecipitation **IC** Immunocytochemistry **IH** Immunohistochemistry (Tissue) **PIA** Peptide Inhibition Assay **BD** Beadlyte® Assay **ChIP-Seq** Chromatin Immunoprecipitation Sequence

SPECIES LEGEND: Ch Chicken **H** Human **M** Mouse **R** Rat **Rb** Rabbit **Ce** *C. elegans* **WR** Most Common Vertebrates

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Additional Research Applications

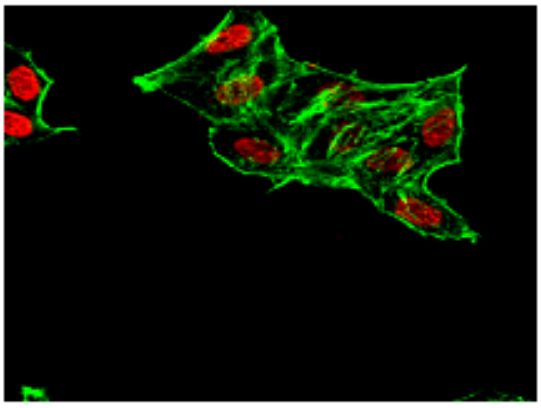
Dot Blot Analysis: A representative lot of this antibody was used by an independent laboratory for DB (Strome and Ahringer Lab, UC Santa Cruz/ University of Cambridge). See Egelhofer, T.A., *et al*. (2011).

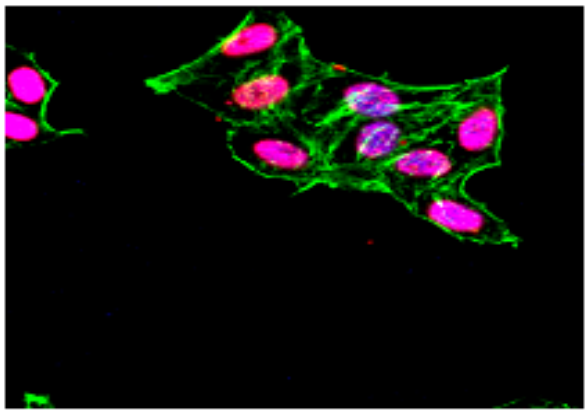
ChIP-Seq Analysis: A representative lot of this antibody was used by two independent laboratories for ChIP-Seq (Strome and Ahringer Lab, UC Santa Cruz/ University of Cambridge and Julie Ahringer Lab, University of Cambridge). See Egelhofer, T.A., *et al*. (2011).

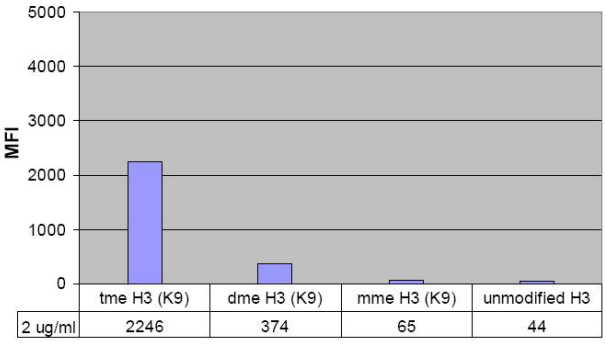
Peptide Inhibition: Specificity was confirmed by the ability of 10 mM of the immunizing peptide to completely abolish detection of histone H3 in immunoblot analysis of HeLa acid extracts. No inhibition of detection was observed by preabsorption of the antibody with 10 mM histone H3 peptide containing mono- or dimethyl-lysine 9, or mono-, di- or trimethyl-lysine 27 modifications (Data not shown).

Immunocytochemistry: Representative lot data. Confocal fluorescent analysis of HeLa cells using anti-Histone pAB (Red). Actin filaments have been labeled with Alexa Fluor 488 -Phalloidin (Green). Nuclear is stained with DAPI (Blue).

Note: Nuclear staining







Beadlyte® Histone Peptide Specificity Assay
Representative lot data.
2 ug/ml of purified anti-dimethyl-Histone H3 (Lys9) was incubated with a cocktail of microspheres conjugated to histone H3 peptides with the following modifications:

1. trimethy-lysine 9
2. dimethyl-lysine 9
3. monomethyl-lysine 9
4. Unmodified H3

Unbound antibody was then removed by filtration. Peptide antibody complexes were incubated with a PE-conjugated anti-rabbit secondary antibody. 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 5% nonfat dry milk (Catalog # 20-200), 0.05% Tween®-20 (TBST-MLK), and 1% BSA for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **1:500 dilution of anti-trimethyl-Histone H3 (Lys9)**, diluted in freshly prepared TBST-MLK for 20 minutes with agitation at room temperature.
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 TBST-MLK for 0.5 hours at room temperature with agitation.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-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).

Acid Extraction of Proteins from HeLa Cells

1. Grow cells to 70% confluency in DMEM supplemented with 10% FBS.
2. Scrape the cells from the plate.
3. Pellet the cells by centrifugation at 200 x g for 10 minutes.
4. Decant the supernatant fraction.
5. Suspend the cells with 10-15 volumes of PBS and centrifuge at 200 x g for 10 minutes.
6. Decant supernatant fraction (PBS wash).
7. Suspend the cell pellet in 5-10 volumes of **lysis buffer**.
8. Add hydrochloric acid to a final concentration of 0.2 M (0.2 N). **Use polypropylene tubes**.
9. Incubate on ice for 30 minutes.
10. Centrifuge at 11,000 x g for 10 minutes at 4°C.
11. Keep the supernatant fraction, which contains the acid soluble proteins, and discard the acid-insoluble pellet.
12. Dialyze the supernatant against 200 mL 0.1 M (0.1 N) acetic acid, twice for 1-2 hours each.
13. Dialyze three times against 200 mL H2O 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
1.5 mM MgCl2
10 mM KCl
*1.5 mM PMSF
*0.5 mM DTT
*1.5 mM PMSF

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

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)
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
17-611	Magna ChIP™ G
17-409	EZ-Magna ChIP™ G
17-610	Magna ChIP™ G
17-408	EZ-Magna ChIP™ G
20-400	Magna GrIP™ Rack (8 well)
12-687	ChIPable Chromatin HeLa
12-704	ChIPable Chromatin 293
12-705	ChIPable Chromatin HT29
17-622	ChIPAb+ Trimethyl-Histone H3 (Lys 27)
17-625	ChIPAb+ Trimethyl-Histone H3 (Lys 9)
17-600	ChIPAb+ CREB
17-601	ChIPAb+ Sp1
17-603	ChIPAb+ ERα
17-604	ChIPAb+ LEF1

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 um IPVH07850
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)