Anti-acetyl-Histone H4

Polyclonal Antibody

Cat. # 06-866

Lot # JBC1885060

pack size: 200 µL

Store at -20ºC

FOR RESEARCH USE ONLY

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, ChIP, IC	H, T, Eu	IgG	a.a. 2-19	Rb	10 kDa	NM_175054

Background

Histone H4 is one of the 5 main histone proteins involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N terminal tail H4 is involved with the structure of the nucleosomes of the 'beads on a string' structure. Acetylation of histone H4 occurs at several different lysine positions in the histone tail and is performed by a family of enzymes known as Histone Acetyl Transferases (HATs).

Presentation

Whole antiserum containing 0.05% sodium azide.

Specificity

Acetylated histone H4, acetylated histone H2B from *Tetrahymena* and weakly cross-reacts with acetylated histone H2B from HeLa cells, may cross-react with other acetylated proteins.

Species Cross-reactivity

Human and *Tetrahymena.* Other species not tested, but expected to cross-react since Histone H4 is well conserved.

Immunogen

KLH-conjugated peptide corresponding to amino acids 2-19 of *Tetrahymena* histone H4 [AGGAcKGGAcKGMGAcKVGAAcKRHS-C], acetylated on lysines 5, 8, 12 and 16.

Molecular Weight

10 kDa

Storage and Handling

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

Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

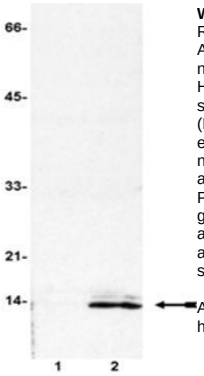
Control

TSA-treated Jurkat cells. For a negative control, perform a no-antibody immunoprecipitation by incubating the supernatant fraction with 60µl of Salmon Sperm DNA/Protein A Agarose- 50% Slurry. Transcriptionally unactivated DNA samples should be prepared as controls for PCR.

Quality Control Testing

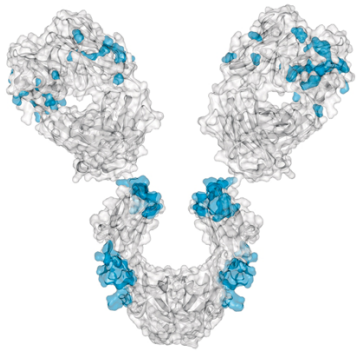
Routinely evaluated by western blot on *Tetrahymena* macronuclei or acid extracted proteins from HeLa cells treated with 5 mM sodium butyrate.

Western Blot Analysis: 1:2000 dilution of this lot detected acetylated histone H4 in acid extracted proteins from HeLa cells treated with 5 mM sodium butyrate. Sodium butyrate, an inhibitor of deacetylases, was used to enhance detection of acetylated histone H4.



Western Blot Analysis
Representative lot data. Acid-extracted proteins from normal HeLa cells (Lane 1) and HeLa cells treated with 5 mM sodium butyrate for 24 hours (Lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-acetyl Histone H4 (1:2000). Proteins were visualized using a goat-anti rabbit secondary antibody conjugated to HRP and a chemi-luminescence detection system.

Arrow indicates acetylated histone H4 (~10 kDa).



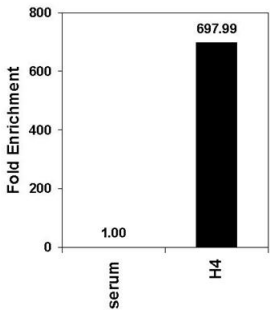
References

1. Dammer, Eric B., *et al.* (2007). *Mol Endocrinol.* 21: 415-38.
2. Sakamoto, A., *et al.* (2004). *Hum Mol Genet.* 13: 819-28.
3. Caretti, G., *et al.* (2003). *J Biol Chem.* 278: 30435-40.
4. Park, S. W. and Wei, L. N. (2003). *J Biol Chem.* 278: 29776-29782.
5. Siegel, P. M., *et al.* (2003). *J Biol Chem.* 278: 35444-35450.
6. Toyota, M., *et al.* (2003). *Proc Natl Acad Sci USA. 100: 7818-23.*

Additional Research Applications

Chromatin Immunoprecipitation:

Representative lot data. 5-10 µL immunoprecipitated transcriptionally active chromatin containing acetylated histone H4 from 2 X 10^6 serum stimulated HeLa cells.



APPLICATION LEGEND: **WB** Western Blotting **ChIP** Chromatin Immunoprecipitation **IP** Immunoprecipitation **IC** Immunocytochemistry **IH** Immunohistochemistry (Tissue)

SPECIES LEGEND: **Eu** Eukaryote **H** Human **M** Mouse **R** Rat **Rb** Rabbit **T** Tetrahymena

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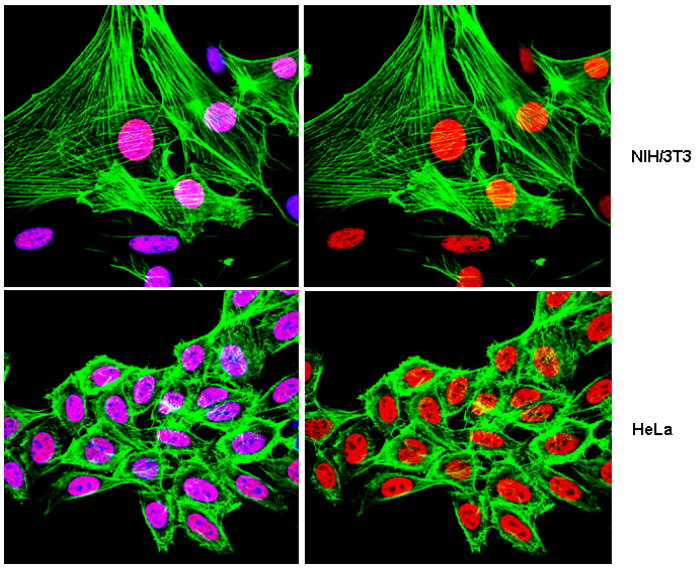
Rev.B/2009-08-26/06-866CA/JM

Additional Research Applications

Immunocytochemistry:

Representative lot data.

Confocal fluorescent analysis of HeLa and NIH/3T3 cells using 06-866 (Red). Actin filaments have been labeled with AlexaFluor®488-Phalloidin (Green). Nucleus is stained with DAPI (Blue). This antibody positively stains the nucleus.



PROTOCOL

Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on acid-extracted protein from cells treated with or without sodium butyrate (see the protocol below) 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 one hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **1:2000 dilution of anti-acetyl Histone H4** in freshly prepared PBS-MLK, at for 3 hours with agitation at room temperature.
4. Wash the nitrocellulose three times with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit IgG conjugated to HRP, 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 three times.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 5 minutes.
8. Rinse the nitrocellulose with 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Acid Extraction of Proteins from Sodium Butyrate Treated 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 sulfuric acid to a final concentration of 0.2 M (0.4N). **Use polypropylene tubes.**
10. Incubate on ice for 30 minutes.
11. Centrifuge at 11,000 x g for 10 minutes at 4℃.
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 (0.1N) 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℃.

Lysis buffer:

10 mM HEPES, pH 7.9
1.5 mM MgCl2
10 mM KCl
*0.5 mM DTT
*1.5 mM PMSF
*Add PMSF and DTT just prior to use of the buffer.

Chromatin Immunoprecipitation

Part A. Optimization of DNA Shearing

Establish optimal conditions required for shearing cross-linked DNA to 200-1000 base pairs in length by following steps 1- 9 below. Vary the power setting and/or the number of 10-second pulses during sonication of the samples. Be sure to keep the sample on ice at all times (the sonication generates heat which will denature the DNA). Check the size of sonicated DNA by gel electrophoresis after reversion of cross-links. Our experience shows DNA is sheared to the appropriate length with 3-4 sets of 10-second pulses using a Cole Parmer, High Intensity Ultrasonic Processor/Sonicator, 50-watt model equipped with a 2 mm tip and set to 30% of maximum power.

Once sonication conditions have been optimized, keep cell number consistent for subsequent experiments. The protocol below for the optimization of DNA Shearing is for one Chip assay (~1 x 10^6 cells per condition).

Note: Steps 3-7 should be done on ice.

1. Stimulate or treat 1 x 10^6 cells on a 10 cm dish as appropriate. (Cells should be treated under conditions for which transcriptional activation of the gene of interest has been demonstrated). Include one extra dish (1 X 10^6 cells) to be used solely for estimation of cell number.
2. 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. (For example, add 270 µL 37% formaldehyde into 10 mL of growth medium on plate).
3. Aspirate medium, removing as much medium as possible. Wash cells twice using ice cold PBS containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride (PMSF), 1 µg/mL aprotinin and 1 µg/mL pepstatin A). Note: Add protease inhibitors to PBS just prior to use. PMSF has a half-life of approximately 30 minutes in aqueous solutions.
4. Scrape cells into conical tube.
5. Pellet cells for 4 minutes at 2000 rpm at 4°C. W arm **SDS Lysis Buffer (Catalog # 20-163)** to room temperature to dissolve precipitated SDS and add protease inhibitors (inhibitors: 1 mM PMSF, 1 µg/mL aprotinin and 1 µg/mL pepstatin A).
6. Resuspend cell pellet in 200 µL of **SDS Lysis Buffer (Catalog # 20-163)** and incubate for 10 minutes on ice. **Note:** The 200 µL of SDS Lysis Buffer is per 1 X 10^6 cells; if more cells are used, the resuspended cell pellet should be divided into 200 µL aliquots so that each 200 µL aliquot contains ~1 X 10^6 cells.
7. Sonicate lysate to shear DNA to lengths between 200 and 1000 basepairs being sure to keep samples ice cold (***Note:*** Once sonication conditions have been optimized following steps 1 to 9, proceed to Part B, step 1 below).
8. Add 8 µL **5 M NaCl (Catalog # 20-159)** and reverse crosslinks at 65°C for 4 hours.
9. Recover DNA by phenol/chloroform extraction and run sample (example 5 µL,10 µL and 20 µL samples) in an agarose gel to visualize shearing efficiency.

Part B. Experimental protocol.

If sonication conditions have been optimized (Part A), complete steps 1 through 7 and continue with the protocol below. For a negative/background control, prepare a sample to use as a no-antibody immunoprecipitation control in step 5 below. Additionally, transcriptionally unactivated DNA samples should be prepared as controls for PCR in section II.

1. Centrifuge samples (part A, step 7) for 10 minutes at 13,000 rpm at 4°C, and add 200 µL of the sonicated cell pellet suspension to a new 2 mL-microcentrifuge tube.
2. Dilute the sonicated cell pellet suspension 10 fold in **ChIP Dilution Buffer (Catalog # 20-153)**, adding protease inhibitors as above. This is done by adding 1800 µL ChIP Dilution Buffer to the 200 µL sonicated cell pellet suspension for a final volume of 2 mL in each immunoprecipitation condition. **Note**: *If proceeding to PCR a portion of the diluted cell pellet suspension 1% (~20 µL) can be kept to quantitate the amount of DNA present in different samples at the PCR protocol, Part B, section II, step 6. This sample is considered to be your input/starting material and needs to have the Histone-DNA crosslinks reversed by heating at 65°C for 4 hours (see section II, step 3.)*
3. To reduce nonspecific background, pre-clear the 2 mL diluted cell pellet suspension with 80 µL of **Salmon Sperm DNA/Protein A Agarose-50% Slurry (Catalog # 16-157)** for 30 minutes at 4°C with agitation.
4. Pellet agarose by brief centrifugation and collect the supernatant fraction.
5. Add the immunoprecipitating antibody (the amount will vary per antibody) to the 2 mL supernatant fraction and incubate overnight at 4°C with rotation. *For a negative control, perform a no-antibody immunoprecipitation by incubating the supernatant fraction with 60 µL of **Salmon Sperm DNA/Protein A Agarose- 50% Slurry (Catalog # 16-157)** for one hour at 4°C with rotation and proceed to st ep 7*.
6. Add 60 µL of **Salmon Sperm DNA/Protein A Agarose Slurry (Catalog # 16-157)** for one hour at 4°C with rotation to collect the antibody/histone complex.
7. Pellet agarose by gentle centrifugation (700 to 1000 rpm at 4°C, ~1 min). Carefully remove the supe rnatant that contains unbound, non-specific DNA. Wash the protein A agarose/antibody/histone complex for 3-5 minutes on a rotating platform with 1 mL of each of the buffers listed in the order as given below:
   - **a.** Low Salt Immune Complex Wash Buffer (Catalog # 20-154), **one wash**
   - **b.** High Salt Immune Complex Wash Buffer (Catalog # 20-155), **one wash**
   - **c.** LiCl Immune Complex Wash Buffer (Catalog # 20-156), **one wash**
   - **d.** TE Buffer (Catalog # 20-157), **two washes.**

After step 7 above, the sample is now a protein A/antibody/histone/DNA complex ready for either an Immunoprecipitation/Western Blot assay (Section I) or Polymerase Chain Reaction (PCR) assay (Section II).

Section I. Immunoprecipitation/Western Blot protocol to detect histone.

1. Following washing of the beads in part B, step 7, immunoprecipitated histones can be analyzed by Western Blot analysis. Add 25 µL of 1X Laemmli buffer per sample and boil for 10 minutes. Load 20 µL per lane and perform western blot procedure as described per appropriate antibody.

Section II. PCR protocol to amplify DNA that is bound to the immunoprecipitated histone.

1. Freshly prepare elution buffer (1%SDS, 0.1 M NaHCO3).
2. Elute the histone complex from the antibody by adding 250 µL elution buffer to the pelleted protein A agarose/antibody/histone complex from step 7d above. Vortex briefly to mix and incubate at room temperature for 15 minutes with rotation. Spin down agarose, and carefully transfer the supernatant fraction (eluate) to another tube and repeat elution. Combine eluates (total volume = ~500 µL).
3. Add 20 µL 5 M NaCl (Catalog # 20-159) to the combined eluates (500 µL) and reverse histone-DNA crosslinks by heating at 65℃ for 4 hours. At this step the sample can be stored and -20℃ and the protocol continued the next day.

Note: Include the input/starting material *(the sample saved from Part B, step 2, which has had the Histone-DNA crosslinks reversed) as well as a transcriptionally - unactivated DNA sample as negative and background controls for the PCR reaction. Previously, a 5µl sample has been used in a nested PCR reaction. However, the amount of sample used per reaction must be determined empirically (e.g., titrate the sample at this step by using 1, 2, 5, or 10µl per PCR reaction)*. If PCR results are poor, complete steps 4, 5 and 6 below to purify the DNA sample. NOTE: Handle the samples carefully, some DNA may be lost during the purification steps.

4. Add 10 µL of 0.5 M EDTA (Catalog # 20-158), 20 µL 1 M Tris-HCl, pH 6.5 (Catalog # 20-160) and 2 µL of 10 mg/mL Proteinase K to the combined eluates and incubate for one hour at 45℃.
5. Recover DNA by phenol/chloroform extraction and ethanol precipitation. Addition of an inert carrier, such as 20 µg glycogen or yeast tRNA, helps visualize the DNA pellet. Wash pellets with 70% ethanol and air dry.
6. Resuspend pellets in an appropriate buffer for PCR or slot-blot reactions. PCR or slot-blot conditions must be determined empirically.

RELATED PRODUCTS (specific)

cat #	description
06-866	Anti-acetyl-Histone H4
06-761	Anti-acetyl-Histone H4 (Lys12)
07-329	Anti-acetyl-Histone H4 (Lys16)
06-759	Anti-acetyl-Histone H4 (Lys5)
04-118	Anti-acetyl-Histone H4 (Lys5), rabbit monoclonal
06-760	Anti-acetyl-Histone H4 (Lys8)
04-557	Anti-acetyl-Histone H4, pan (Lys 5,8,12)
07-213	Anti-dimethyl-Histone H4 (Arg3)
05-672	Anti-dimethyl-Histone H4 (Lys20), clone 6G7/H4
05-734	Anti-dimethyl-Histone H4 (Lys79), clone ER133
05-754	Anti-di-tri-methyl-Histone H4 (Lys20), clone AW317
07-596	Anti-Histone H4 (citrulline 3)
05-858	Anti-Histone H4, pan
06-946	Anti-hyperacetylated Histone H4 (Penta)
05-735	Anti-monomethyl-Histone H4 (Lys20), clone NL314
04-079	Anti-trimethyl-Histone H4 (Lys20), rabbit monoclonal
17-211	Acetyl-Histone H4 Antibody Set
17-229	Acetyl-Histone H4 Immunoprecipitation (ChIP) Assay Kit
17-212	Acetyl-Histone H4 Peptide Pack
17-217	Acetyl-Histone H4 Site Specificity Pack
12-348	Goat Anti-Rabbit IgG
17-500	Catch and Release Reversible Immunoprecipitation System
16-266	Protein G Agarose Fast Flow 10 mL
16-125	Protein A-Agarose 10 mL

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)