

Anti-Acetyl Histone H4, ChIPs Grade

(rabbit antiserum)

Catalog # 06-866

Lot # 18784

Immunogen: KLH-conjugated peptide [AGG_{Ac}KGG-_{Ac}KGMG_{Ac}KVGA_{Ac}KRHSC] corresponding to amino acids 2-19 of *Tetrahymena* histone H4.

Specificity: Recognizes acetylated histone H4 of approximately 10kDa. Cross-reacts with acetylated histone H2B from *Tetrahymena* and weakly cross-reacts with acetylated histone H2B from HeLa cells. May cross-react with other acetylated proteins.

Cross-reactivity: Human and *Tetrahymena*. Other species not tested.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

Formulation: 200 μ l of whole antiserum containing 0.05% sodium azide. Frozen solution.

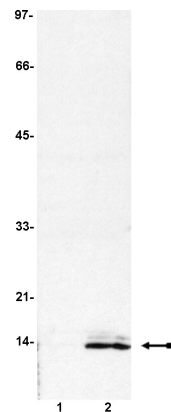
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

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

Chromatin Immunoprecipitation: 5 μ l of a previous lot immunoprecipitated transcriptionally active chromatin containing acetylated histone H4 from 2 X 10⁶ serum stimulated HeLa cells.

Immunocytochemistry: Not recommended. Use Catalog # 06-598.



Immunoblot Analysis.

Acid-extracted proteins from normal HeLa cells (Lane 1) and HeLa cells treated with 5mM 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 (10kDa).

Background: The amino-terminal tails of core histones (H2A, H2B, H3 and H4) are targets for post-translational modifications that include acetylation of the ϵ -amino group of specific lysine residues. Histone acetylation is believed to be a hallmark of transcriptionally active chromatin. Acetylation of basic histones is thought to weaken the histone:DNA ionic interactions thereby facilitating access of the transcriptional machinery. Lysines 5, 8, 12 and 16 of human histone H4 are the targets of acetylation. The tri- and tetra-acetyl isoforms of histone H4 are typically found in transcriptionally active chromatin. This antibody can be used to immunoprecipitate transcriptionally active genes from *Tetrahymena*, yeast and mammalian cells in a chromatin immunoprecipitation (ChIP) assay.

Application References:

1. Alberts, A. S., *et al.*, Cell **92**: 475-487, 1998.
2. Braunstein, M., *et al.*, Genes & Devel. **7**: 592-604, 1993.
3. Lin, R., *et al.*, J. Cell Biol. **108**: 1577-1588, 1989.
4. Perry, C.A., *et al.*, Biochem. **32**: 13605-13614, 1993.

Immunoblot Protocol

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 (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1:2000 dilution of a-acetyl Histone H4, ChIPs Grade** in freshly prepared PBS-MLK, overnight with agitation at 4°C.
4. Wash the nitrocellulose three times with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-rabbit IgG 1:3000 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 in water for 10 minutes. 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 (100mM sterile stock solution), which inhibits histone deacetylases, to a final concentration of 5mM 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 0.2M (0.4N). **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 200ml 0.1M (0.1N) acetic acid, twice for 1-2 hours each.
14. Dialyze three times against 200ml H₂O for 1hour, 3 hours, and overnight, respectively. The protein can be quantified and lyophilized or stored at -70°C.

Lysis buffer:

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

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

Chromatin Immunoprecipitation Protocol

Perform a mock experiment (steps 1-5) to establish optimal conditions required to shear cross-linked DNA to 200-1000 basepairs in length. Vary the power setting and/or the number of 10 second pulses during sonication of the samples. Chill samples between pulses. Check the size of sonicated DNA by gel electrophoresis after reversion of cross-links (step 14). Our experience shows DNA is sheared to the appropriate length with 3 sets of 10 second pulses using a Vibra cell sonicator equipped with a 2mm tip and set to 30% of maximum power.

1. Stimulate or treat $0.5-2 \times 10^6$ cells on 10cm dish as appropriate.
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. Seal culture vessels if returning the cultures to an incubator containing other cells.
3. Aspirate medium, wash and scrape cells with ice cold PBS containing protease inhibitors (1mM phenylmethylsulfonyl fluoride (PMSF), 1µg/ml aprotinin and 1µg/ml pepstatin A). We recommend using PMSF stock solutions less than 1 month old and adding PMSF to the buffer just prior to use because PMSF has a half life of about 30 minutes in aqueous solutions.
4. Pellet cells for 4 minutes at 700 x g at 4°C. Resuspend cell pellet in 200µl SDS lysis buffer (1% SDS, 10mM EDTA, 50mM Tris-HCl, pH 8.1) 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 4°C in a microcentrifuge.
6. Dilute supernatant fraction 10 fold in IP buffer (0.01% SDS, 1.1% TritonX-100, 1.2mM EDTA, 16.7mM Tris, pH 8.1, 16.7mM NaCl and protease inhibitors). Keep a portion of this chromatin solution (1%) to check the amount of input DNA present in different samples before immunoprecipitation.
7. To reduce nonspecific background, pre-clear the chromatin solution with 80µl of a 50% protein A sepharose slurry containing 20µg sonicated salmon sperm DNA and 1mg/ml BSA in TE (10mM Tris, 1mM EDTA, pH 8.0) for 30 minutes at 4°C with agitation.
8. Pellet beads by a brief centrifugation and collect supernatant fraction.
9. Add **5ml of anti-acetyl Histone H4, ChIPs Grade** to 1ml of chromatin solution (supernatant fraction of step 8) and incubate overnight at 4°C with rotation. Save the other 1ml of chromatin solution for a no-antibody control.
10. Collect immune complexes with 60µl 50% protein A sepharose slurry containing 20µg sonicated salmon sperm DNA and 1mg/ml BSA in TE for 1 hour at 4°C with rotation.
11. Pellet beads by centrifugation and wash five times using the sequence of buffers listed below. Use 1ml of buffer per wash, 3-5 minutes each:
 - a) 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 150mM NaCl.
 - b) 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 500 mM NaCl.
 - c) 0.25M LiCl, 1% NP40, 1% sodium deoxycholate, 1mM EDTA, 10mM Tris-HCl, pH 8.1.
 - d) TE, pH 8.0.
 - e) TE, pH 8.0.
13. Elute immune complexes by adding 250µl 1% SDS in 0.1M NaHCO₃ to 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 20µl 5M NaCl to the combined eluates and reverse crosslinks at 65°C for 4 hours. **NOTE:** 5µl of this material can be used in nested-PCR to determine if the gene/promotor of interest was immunoprecipitated.
15. Add 10µl of 0.5M EDTA, 20µl 1 M Tris-HCl, pH 6.5, and 2µl of 10mg/ml Proteinase K to the eluate and incubate for 1 hour at 45°C.
16. Recover DNA by phenol/chloroform extraction and ethanol precipitation using 20µg glycogen as a carrier.
17. Detect specific sequences from immunoprecipitated and input DNA by quantitative PCR or slot-blot. Conditions must be determined empirically