
Certificate of Analysis

Acetyl-Histone H4 Immunoprecipitation (ChIP) Assay Kit

Catalog # 17-229

Lot # 20731

Kit Components

Anti Acetyl-Histone H4, ChIP Grade, Catalog # 06-866, Lot # 20667. One vial containing **200ml** of rabbit antiserum with 0.05% sodium azide. Frozen solution.

Salmon Sperm DNA/Protein A Agarose, Catalog # 16-157C, Lot # 20799. One vial containing **1.5ml** packed beads with **600mg** sonicated salmon sperm DNA, 1.5mg BSA, ~4.5mg recombinant protein A. Provided as a 50% gel slurry for a final volume of 3ml. Suspended in 10mM Tris-HCl, pH 8.0, 1mM EDTA, containing 0.05% sodium azide. Liquid suspension.

SDS Lysis Buffer, Catalog # 20-163. One vial containing **10ml** of 1% SDS, 10mM EDTA, 50mM Tris-HCl, pH 8.1.

ChIP Dilution Buffer, Catalog # 20-153. Two vials, each containing **25ml** of 0.01% SDS, 1.1% Triton X-100, 1.2mM EDTA, 16.7mM Tris-HCl, pH 8.1, 167mM NaCl.

Low Salt Immune Complex Wash Buffer, Catalog # 20-154. One vial containing **25ml** of 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 150mM NaCl.

High Salt Immune Complex Wash Buffer, Catalog # 20-155. One vial containing **25ml** of 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 500mM NaCl.

LiCl Immune Complex Wash Buffer, Catalog # 20-156. One vial containing **25ml** of 0.25M LiCl, 1% NP40, 1% deoxycholate, 1mM EDTA, 10mM Tris-HCl, pH 8.1.

1X TE Buffer, Catalog # 20-157. Two vials, each containing **25ml** of 10mM Tris-HCl, 1mM EDTA, pH 8.0.

5M NaCl, Catalog # 20-159. One vial containing **500ml** of 5M NaCl.

0.5M EDTA, Catalog # 20-158. One vial containing **250ml** of 0.5M EDTA, pH 8.0.

1M Tris-HCl, pH 6.5, Catalog # 20-160. One vial containing **500ml** of 1M Tris-HCl, pH 6.5.

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

Kit Description

Quantity: 20 chromatin immunoprecipitation (ChIP) assays and 2 negative controls per kit.

Storage and Stability: Stable for 1 year at 4°C from date of shipment.

Use: The assay kit contains reagents optimized for immunoprecipitation of transcriptionally active chromatin from mammalian cells using anti-Acetyl-Histone H4, ChIP grade rabbit antiserum. Detection of the gene or promoter of interest in immunoprecipitated chromatin must be empirically determined by the researcher. Quantitative PCR or Southern slot-blot analysis, using promoter specific primers or probe, is recommended.

Technical Information for Kit Components

Anti-Acetyl-Histone H4, ChIP Grade (rabbit polyclonal antiserum)

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

Specificity: Recognizes acetylated histone H4, Mr ~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.

Species Cross-reactivity: Human, mouse and *Tetrahymena*; broad species cross-reactivity is expected.

Storage and Stability: Stable for six months at 4°C or 2 years at -20°C. Aliquot to avoid repeated freezing and thawing).

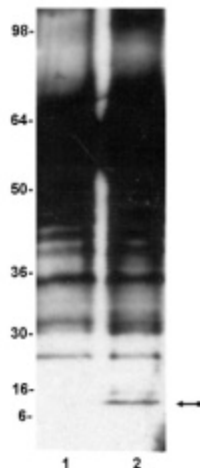
Background: The amino-terminal tails of core histones (H2A, H2B, H3 and H4) are targets for post-translational modifications that include acetylation of the ε-amino group of specific lysine residues. Histone acetylation is believed to be a hallmark of transcriptionally active chromatin. Acetylation of basic histones may 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 a variety of 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. Perry, C.A., *et al.*, Biochem. **32**: 13605-13614, 1993.
4. Lin, R., *et al.*, J. Cell Biol. **108**: 1577-1588, 1989.

Quality Control Testing

Chromatin Immunoprecipitation: 5-10µl of Anti-acetyl Histone H4, ChIP grade immunoprecipitated acetylated histone H4 as determined by subsequent immunoblot analysis using 1µg/ml anti-acetyl Histone H4 antibody (Catalog # 06-598) to detect histone from 1 X 10⁶ 3T3/A431 cells treated overnight with 5mM sodium butyrate.



Chromatin Immunoprecipitation

Representative blot from a previous lot. Anti-acetyl Histone H4, ChIP grade, was used to immunoprecipitate chromatin from lysates of quiescent 3T3/A31 cells (lane 1) or sodium butyrate treated 3T3/A31 cells (lane 2). The immunoprecipitates were resolved by electrophoresis, transferred to nitrocellulose and probed with 1µg/ml anti-acetyl Histone H4 (Catalog # 06-598). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates acetylated H4.

Other components required but not included as part of kit are:

Reagents

- Cells, stimulated or treated as appropriate for the experimental system
- 37% Formaldehyde
- PBS
- Dry ice
- Elution buffer: 1% SDS, 0.1M NaHCO₃
- Molecular Biology grade Proteinase K
- Glycogen or tRNA
- 50% Phenol/50% Chloroform (containing 1% isoamyl alcohol)
- > 95% Ethanol
- 70% Ethanol

Equipment

- vortex mixer
- rotating wheel/platform
- shaking incubator
- timer
- variable volume (5-1000µl) pipetters + tips
- microfuge
- variable temperature water bath
- humidified 37°C incubator
- cell scraper
- sonicator

Safety Warnings and Precautions: Formaldehyde should be used in a certified fume hood. Culture dishes containing formaldehyde should be placed in a sealed bag if returned to a humidified 37°C incubator to prevent exposing other cells in the incubator to formaldehyde. Ear protection must be worn during sonication. All chemicals should be considered potentially hazardous and handled with the principles of good laboratory practice.

Chromatin Immunoprecipitation Protocol

Perform a mock experiment (steps 1-5) to establish optimal conditions required to shear cross-linked DNA to 200-1000 base pairs 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 x 10⁶ cells on 10cm dish as appropriate.¹ (Refer to Technical Tips below)
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.² 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 one month old and adding PMSF to the buffer just prior to use since 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. Warm **SDS Lysis Buffer (Catalog # 20-163)** to room temperature to dissolve precipitated SDS and add protease inhibitors (inhibitors: 1mM PMSF, 1µg/ml aprotinin and 1µg/ml pepstatin A). Resuspend cell pellet in 200µl **SDS Lysis Buffer (Catalog # 20-163)** for 10 minutes on ice.³
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 **ChIP Dilution Buffer (Catalog # 20-153)**. Add protease inhibitors as above. Keep a portion of this chromatin solution (1%) to quantitate the amount of DNA present in different samples before immunoprecipitation.

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7. To reduce nonspecific background, pre-clear the chromatin solution with 80µl of **Salmon Sperm DNA/Protein A Agarose Slurry (Catalog # 16-157)** 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, ChIP Grade (Catalog # 06-866)** 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 of **Salmon Sperm DNA/Protein A Agarose Slurry (Catalog # 16-157)** for one hour at 4°C with rotation.
11. Prepare elution buffer (1%SDS, 0.1M NaHCO₃).⁴
12. Pellet beads by centrifugation.⁵ Wash the beads for 3-5 minutes on a rotating platform with 1ml of each of the buffers listed below:⁶
 - Low Salt Immune Complex Wash Buffer (Catalog # 20-154)**
 - High Salt Immune Complex Wash Buffer (Catalog # 20-155)**
 - LiCl Immune Complex Wash Buffer (Catalog # 20-156)**
 - 1X TE (Catalog # 20-157)**
 - 1X TE (Catalog # 20-157)**
13. Elute immune complexes by adding 250µl elution buffer 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 (Catalog # 20-159)** to the combined eluates and reverse crosslinks at 65°C for 4 hours.⁷
15. Add 10µl of **0.5M EDTA (Catalog # 20-158)**, 20µl **1M Tris-HCl, pH 6.5 (Catalog # 20-160)** and 2µl of 10mg/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 20µg glycogen or yeast RNA is suggested. Wash pellets with 70% ethanol and allow to air dry.
17. Resuspend pellets in an appropriate buffer or water. Detect specific sequences from no antibody and immunoprecipitated samples by quantitative PCR or slot-blot. Include input and unbound DNA samples as controls. Conditions for PCR amplification must be determined empirically.

Technical Tips for ChIP Protocol

1. Cells should be treated under conditions for which transcriptional activation of the gene of interest has been demonstrated.
2. The formaldehyde crosslinking conditions listed in this protocol have been optimized for the anti-acetyl histone H4, ChIPs Grade antibody (Catalog # 06-866). For other antibodies, crosslinking conditions (duration and temperature) must be empirically determined.
3. An alternate lysis procedure can be used which may reduce background in the no antibody control immunoprecipitation.
 - Resuspend cell pellet in 200µl 5mM Pipes pH 8.0, 85mM KCl, 0.5% NP40 containing protease inhibitors.
 - Place on ice for 10 minutes. Pellet by centrifugation (5 minutes at 5000 rpm). Resuspend pellet in 200µl 1% SDS, 10mM EDTA, 50mM Tris-HCl, pH 8.1 containing protease inhibitors. Incubate on ice for 10 minutes.
4. Use of freshly prepared elution buffer is recommended; however, elution buffer prepared in advance may be used if it is no more than 1 month old.
5. Aliquots can be removed at this point which represent unbound DNA.
6. Following washing of the beads, immunoprecipitated histones can be assessed by immunoblot analysis after boiling the samples in Laemmli buffer for 10 minutes.
7. Eluted material can be used as template in PCR reactions. We have successfully used 5µl of this material in a 100µl nested-PCR reaction.