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Certificate of Analysis

199 Saranac Avenue • Lake Placid, NY 12946
Technical Support: T: 800 548-7853 • F: 518 523-4513
email: techserv@upstatebiotech.com
Sales Department: T: 800 233-3991 • F: 617 890-7738
Licensing Dept.: 800 310-4659
www.upstatebiotech.com

Acetyl-Histone H3 ChIP Assay Kit

(for mammalian cells)
Catalog # 17-245
Lot # 18696

Kit Components

Anti Acetylated-Histone H3, Catalog # 06-599, Lot # 17300, see page two for more information. One vial containing **200µg** of protein A purified IgG in **200µl** of 0.1M Tris-glycine, pH 7.4, 0.15M sodium chloride with 0.05% sodium azide.

Salmon Sperm DNA/Protein A Agarose, Catalog # 16-157, Lot # 18110. Three vials, each containing **500µl** packed beads, 100µg sonicated salmon sperm DNA and 500µg BSA. Provided as a 50% gel slurry, in 1X TE buffer containing 0.05% sodium azide for a final volume of 1ml.

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 **500µl** of 5M NaCl.

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

1 M Tris-HCl, pH 6.5, Catalog # 20-160. One vial containing **500µl** 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 6 months 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 H3. 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 promotor specific primers or probe, is recommended.

Technical Information for Kit Components

Anti-Acetyl-Histone H3
(rabbit polyclonal IgG)

Immunogen: KLH conjugated peptide (ARTKQTAR[K*]STGG[K*]APRKQLC where K* is acetylated) corresponding to amino acids 1-21 of *Tetrahymena* histone H3.

Specificity: Recognizes and is specific for acetylated histone H3.

Species Cross-reactivity: Human and *Tetrahymena*. Although other species not tested, a broad species cross reactivity is expected.

Physical Form: Liquid. Stable for six months at 4ºC from date of shipment or 2 years at -20ºC. Aliquot to avoid repeated freezing and thawing.

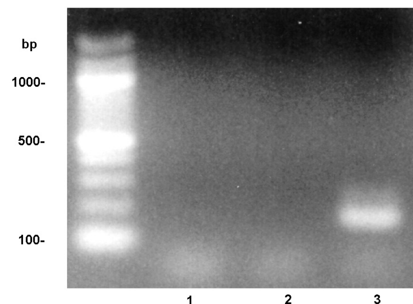
Background: Histones in the nucleosome core (H2A, H2B, H3 and H4) possess a positively charged unfolded amino terminal domain that is a target for post-translational modifications. One recently described modification is the acetylation of the ε-amino group of lysine residues regulated by an emerging family of enzymes called histone acetyl transferases (HATs) and deacetylases that exhibit specificity for different lysine residues within a particular histone. Histone H3 is acetylated at positions 9, 14, 18, and 23. Acetylation of basic histones may weaken the histone:DNA ionic interactions thereby facilitating access by the transcriptional machinery. This antibody can be used to immunoprecipitate transcriptionally active genes from a variety of cells in a chromatin immunoprecipitation (ChIP) assay.

Application References:

1. Luo, R.X.., *et al*., Cell. 92: 463-473, 1998
2. Braunstein, M., *et al*., Mol. Cell. Biol. **16**: 4349-4356, 1996.
3. Braunstein, M., *et al*., Genes & Devel. **7**: 592-604, 1993.
4. Alberts, A. S., *et al*., Cell. 92: 475-487, 1998

Quality Control Testing

Chromatin Immunoprecipitation: 5µl of Anti-Acetyl Histone H3 immunoprecipitated transcriptionally active chromatin containing acetylated histone H3 from 2 X 10^6 serum stimulated 3T3/A31 cells.



PCR Analysis of Chromatin Immunoprecipitates:
Sheared chromatin from mouse 3T3/A31 cells was immuno-precipitated using 5µl of **anti-acetyl Histone H3** (lane 3) or no antibody (lane 2) and subjected to PCR amplification with c-fos promoter specific probes, followed by agarose gel electro-phoresis. Lane1: No DNA PCR negative control.

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 $NaHCO_3$
- 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 37ºC 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 37ºC 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 10^6 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 37ºC.[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 4ºC. 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.

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 4ºC with agitation.
8. Pellet beads by a brief centrifugation and collect supernatant fraction.
9. Add **5ml of anti-acetyl Histone H3 (Catalog # 06-599)** to 1ml of chromatin solution (supernatant fraction of step 8) and incubate overnight at 4ºC 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 4ºC with rotation.
11. Prepare elution buffer (1%SDS, 0.1M $NaHC0_3$).[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:

    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 65ºC for 4 hours.[6]
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 45ºC.
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 H3, antibody (Catalog # 06-599). 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. Eluted material can be used as template in PCR reactions. We have successfully used 5µl of this material in a 100µl nested-PCR reaction.