

Certificate of Analysis

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Anti-dimethyl-Histone H3 (Lys4)

(rabbit antiserum)

Catalog # 07-030

Lot # DAM1503382

Immunogen: KLH conjugated synthetic peptide (ART_{Me2}KQTAR-GC) corresponding to amino acids 1-8 of human Histone H3.

Formulation: 200µl of rabbit polyclonal antiserum containing 0.05% sodium azide and 30% glycerol. Liquid at -20°C.

Specificity: Recognizes dimethylated Histone H3 (Lys4), Mr 17kDa. Specificity demonstrated by ELISA using methylated peptides.

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

Species Cross-reactivity: Human and tetrahymena. Broad species cross-reactivity is expected.

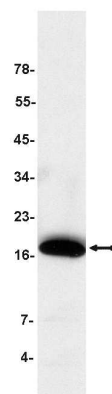
**FOR RESEARCH USE ONLY
 NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 1:2000-1:10,000 dilution of this lot detected methylated Histone H3 in acid extracted proteins from HeLa cells. 1:2000-1:5000 dilution of a previous lot detected methylated Histone H3 in acid extracted proteins from HeLa and tetrahymena cells.

Chromatin Immunoprecipitation: 5µl of a previous lot immunoprecipitated methylated Histone H3 from 1 x 10⁶ 3T3/A31 cells.

Immunocytochemistry: 1:500 dilution of a previous lot showed positive immunostaining for methylated Histone H3 in L6 cells fixed with 4% paraformaldehyde and permeabilized using 0.1% Triton X-100.



Immunoblot Analysis

Representative blot from a previous lot. HeLa cell acid precipitate was resolved by electrophoresis, transferred to nitrocellulose and probed with a 1:5000 dilution of anti-dimethyl Histone H3 (Lys4). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates methylated Histone H3 (17kDa).

Application References:

1. Kohlmaier, A., *et al.*, *PLoS Biol.* **2**: E171, 2004 (Epub).
2. Boggs, B., *et al.*, *Nature Genetics* **30**: 73-76, 2002.

General Reference:

3. Strahl, B.D., *et al.*, *Proc. Natl., Acad. Sci. USA* **96**: 14967-14972, 1999.

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Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) 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 20-30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **1:2000-1:10,000 dilution of anti-dimethyl-Histone H3 (Lys4)**, diluted in freshly prepared PBS-MLK overnight with agitation at 4 $^{\circ}$ C.
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 PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-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).

Immunocytochemistry Protocol

1. Place a previously autoclaved, 13mm circular glass coverslip in the well of a 24 well plate.
2. Plate approximately 1ml of cell suspension into each well. Incubate 24 hours in a 37 $^{\circ}$ C CO₂ incubator.
3. Aspirate media from wells.
4. Add fix 250 μ l-400 μ l (4%paraformaldehyde) in PBS for 10 minutes at room temperature.
5. Permeabilize cells with 0.1% Triton X-100 for 3 minutes.
6. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
7. Cover cells with 400 μ l of 1% BSA in PBS and incubate for 1 hour at room temperature.
8. Wash the cells with PBS for 15 minutes.
9. Incubate the cells with **1:500 anti-dimethyl-Histone H3 (Lys4)** in 1% BSA in PBS and incubate for 2 hours at room temperature.
10. Wash the cells twice with PBS for 5 minutes.
11. Incubate the cells in the dark with a 1:500 dilution of goat anti-rabbit IgG fluorescein conjugated secondary antibody in 1% BSA in PBS for 1 hour at room temperature.
12. Wash the cells three times with PBS.
13. Aspirate well dry.
14. Clean a glass slide with AlconoxTM and water, follow with a rinse in 70% ethanol, dry using a KimwipeTM.
15. Place a drop of Aqua Poly-MountTM mounting media on cleaned slide.
16. Using forceps or a 26-gauge needle with the tip bent, and forceps, retrieve the glass coverslip from the well and place it cell side down on top of the drop of mounting media.
17. Let dry at room temperature, seal edge if desired and examine the cells under a fluorescent microscope.

Chromatin Immunoprecipitation Protocol

*Required Solutions

Protease Inhibitors: 1mM phenylmethylsulfonyl fluoride (PMSF), 1µg/ml aprotinin and 1µg/ml pepstatin A. We recommend using a PMSF stock solution less than one month old and add PMSF to the buffer just prior to use since PMSF has a half-life of about 30 minutes in aqueous solutions.

SDS Lysis Buffer (Catalog # 20-163): 1% SDS, 10mM EDTA, 50mM Tris, pH 8.1.

ChIP Dilution Buffer (Catalog # 20-153): 0.01% SDS, 1.1% Triton X-100, 1.2mM EDTA, 16.7mM Tris, pH 8.1, 167mM NaCl.

1. 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.
2. Aspirate medium, wash and scrape cells with ice cold PBS containing protease inhibitors*.
3. Pellet cells for 4 minutes at 700 x g at 4°C.
4. Warm SDS Lysis Buffer* to room temperature to dissolve precipitated SDS and add protease inhibitors. Resuspend cell pellet in 200µl SDS Lysis Buffer* 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 ChIP Dilution Buffer* with protease inhibitors added. 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 (Catalog # 16-157) for 30 minutes at 4°C with agitation.
8. Pellet beads by a brief centrifugation and collect supernatant fraction.
9. Add **5µl of anti-dimethyl Histone H3** to 1ml of chromatin solution (supernatant fraction of step 7) 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 (Catalog # 16-157) for one hour at 4°C with rotation.
11. Prepare elution buffer (1%SDS, 0.1M NaHCO₃).
12. Pellet beads by centrifugation and wash five times, for 3-5 minutes per wash, using the sequence of buffers listed below. Use 1ml of each buffer per wash.
 - a) Low Salt Immune Complex Wash Buffer (Catalog # 20-154): 150mM NaCl, 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1. **One wash.**
 - b) High Salt Immune Complex Wash Buffer (Catalog # 20-155): 500 mM NaCl, 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1. **One wash.**
 - c) LiCl Immune Complex Wash Buffer (Catalog # 20-156): 0.25M LiCl, 1% NP40, 1% sodium deoxycholate, 1mM EDTA, 10mM Tris-HCl, pH 8.1. **One wash.**
 - d) TE Buffer (Catalog # 20-157): 0.10mM Tris-HCl, 1mM EDTA, pH 8.0. **Two washes.**
13. **Elute immune complexes by adding 250µl elution buffer (see step 11) to the 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.
15. Add 10µl of 0.5M EDTA, 20µl 1M Tris-HCl, pH 6.5, 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 slow 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.

**Following washing of the beads, immunoprecipitated histone can be assessed by immunoblot analysis after boiling of the samples in Laemmli buffer for 10 minutes.