Anti-monomethyl-Histone H3 (Lys4)

Polyclonal Antibody

Cat. # 07-436

Lot # 2474976

pack size: 200 µg
Concentration: 1 mg/mL

Store at 2-8ºC

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, DB, PIA, ICC, ChIP-Sq	H, WR	IgG	N/A	Rb	17 kDa	NM_003493

Background

Histone H3 is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N-terminal tail, H3 is involved with the structure of the nucleosomes of the 'beads on a string' structure. The N-terminal tail of histone H3 protrudes from the globular nucleosome core and can undergo several different types of epigenetic modifications that influence cellular processes. These modifications include the covalent attachment of methyl or acetyl groups to lysine and arginine amino acids and the phosphorylation of serine or threonine.

Presentation

Purified rabbit IgG in buffer containing 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide. Store at 2-8°C.

Specificity

Recognizes monomethyl-histone H3 (Lys4), Mr 17kDa.

Species Cross-reactivity

Human. Broad species to cross-reactivity is expected.

Immunogen

KLH-conjugated, synthetic peptide containing the sequence ART[$_{me}$K]…, in which $_{me}$K corresponds to monomethyl-lysine at residue 4 of human histone H3.

Molecular Weight

17 kDa

Method of Purification

Protein A purified

Storage and Handling

Stable for 1 year at 2-8ºC from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution.

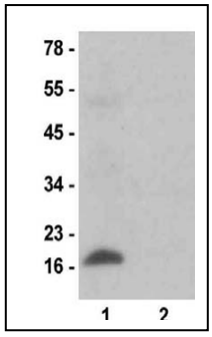
Control

HeLa cell extract

Quality Control Testing

Evaluated by Western Blot in acid extracted proteins from HeLa cells.

Western Blot Analysis: 0.5-2 µg/mL of this lot detected methylated histone H3 in acid extracted proteins from HeLa cells



Western Blot Analysis:
Representative Lot Data
HeLa acid extract (lane 1) and recombinant histone H3 (lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-monomethyl-Histone H3 (Lys4) (0.5µg/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Monomethyl-histone H3 (Lys4), (~17kDa).

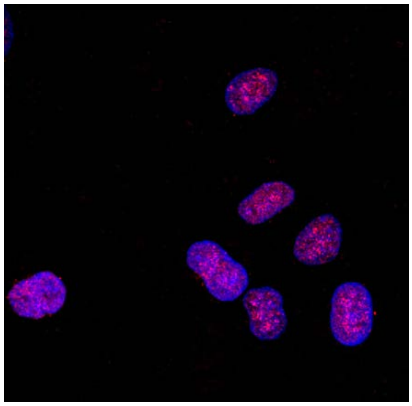
Additional Research Applications

Immunocytochemistry: Reported by an independent laboratory

References

1. Egelhofer, T.A., *et al*. (2011). *Nat Struct Mol Biol*. 18(1):91-93.
2. Strahl, B.D., *et al* (1999). *PNAS*. 96: 14967-14972.
3. Pencovich, N., *et al*. (2011). *Blood.* 117: e1 - e14.

ChIP-Seq Analysis: A representative lot of this antibody was used by an independent laboratory for ChIP-Seq (Bing Ren Laboratory, UC San Diego) See Egelhofer, T.A., *et al.* (2011). See Pencovich, N., *et al.* (2011).



Immunocytochemistry Analysis:
Representative lot data.
Confocal fluorescent analysis of HeLa cells using a 1:2000 dilution of Cat. No. 07-436, Anti-monomethyl Histone H3 (Lys4) Antibody and a Donkey anti-rabbit secondary antibody conjugated to Cy3 (Red). Nucleus is stained with DAPI (Blue). This antibody positively stains the nucleus.

APPLICATION LEGEND: **WB** Western Blotting **IP** Immunoprecipitation **ICC** Immunocytochemistry **FC** Flow Cytometry **IF** Immunofluorescence **IHC** Immunohistochemistry (Tissue) **IHC(P)** Immunohistochemistry (Paraffin)
SPECIES LEGEND: **H** Human **M** Mouse **R** Rat **Rb** Rabbit **WR** Most Common Vertebrates **( )** Predicted Reactivity
Please visit www.millipore.com for additional product information, test data and references.

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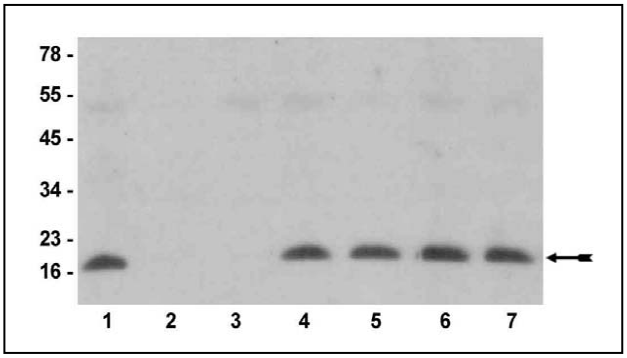
Additional Research Applications

Peptide Inhibition: Specificity was confirmed by the ability of 1 µM of the immunizing peptide to completely abolish detection of histone H3 in immunoblot analysis of HeLa acid extracts. (see figure, lane 3). No competition was observed with peptides containing dimethyl-lysine 4, trimethyl-lysine 4, mono-methyllysine 4 or monomethyl-lysine 9 or 27.



Western Blot Analysis:
Representative lot data.
HeLa acid extract (lane 1 and lanes 3 through 7) and recombinant histone H3 (lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-monomethyl-Histone H3 (Lys4) (0.5µg/mL; lanes 1 and 2) or probed with antibody at the same concentration pre-incubated with 1µM histone H3 peptides with the following modifications:

Lane 3: monomethyl-lysine 4
Lane 4: dimethyl-lysine 4
Lane 5: trimethyl-lysine 4
Lane 6: monomethyl-lysine 9
Lane 7: monomethyl-lysine 27

Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates monomethyl-histone H3 (Lys4), (~17kDa).

PROTOCOL

Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on an acid-extracted protein sample (see protocol below) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200), 1% BSA and 0.05% Tween 20 (TBST-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with 0.5-2 µg/mL of anti-monomethyl-Histone H3 (Lys4), diluted in freshly prepared TBST-MLK 1hours with agitation at room temperature.
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 TBST-MLK for 30 minutes at room temperature with agitation.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.

Use detection method of choice (enhanced chemiluminescence was used).

Acid Extraction of Proteins from HeLa Cells

1. Scrape the cells from the plate.

Pellet the cells by centrifugation at 200 x g for 10 minutes.

2. Decant the supernatant fraction.
3. Suspend the cells with 10-15 volumes of PBS and centrifuge at 200 x g for 10 minutes.
4. Decant supernatant fraction (PBS wash).
5. Suspend the cell pellet in 5-10 volumes of lysis buffer.
6. Add hydrochloric acid to a final concentration of 0.2M (0.2N). Use polypropylene tubes.
7. Incubate on ice for 30 minutes.
8. Centrifuge at 11,000 x g for 10 minutes at 4°C.
9. Keep the supernatant fraction which contains the acid soluble proteins and discard the acid-insoluble pellet.
10. Dialyze the supernatant against 200 mL 0.1M (0.1N) acetic acid, twice for 1-2 hours each.
11. 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°C.
12. **Lysis buffer:**
    10mM HEPES, pH 7.9 *0.5mM DTT
    1.5mM MgCl2 *1.5mM PMSF
    10mM KCl
    *Add PMSF and DTT just prior to use of the buffer.