

# Anti-trimethyl-Histone H3 (Lys4), clone MC315

Monoclonal Antibody

Cat. # 04-745

Lot # 2462884

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

pack size: 100 µL

Store at -20°C



## Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, ChIP, BD, DB, ChIP-Seq	H	IgG	N/A	Rb	17 kDa	NP_003484

### 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

Cultured supernatant in 0.05% sodium azide.

### Specificity

Histone H3 containing trimethyl-lysine 4 and, to a lesser extent, dimethyl-lysine 4.

### Species Cross-reactivity

Human. Broad species cross-reactivity is expected.

### Immunogen

BSA-conjugated, synthetic peptide containing the sequence ...RT<sub>me3</sub>KQT... in which <sub>me3</sub>K corresponds to trimethyl-lysine 4 of human histone H3.

### Storage and Handling

Stable for 1 year at -20°C from date of receipt.  
Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C.  
**Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.**

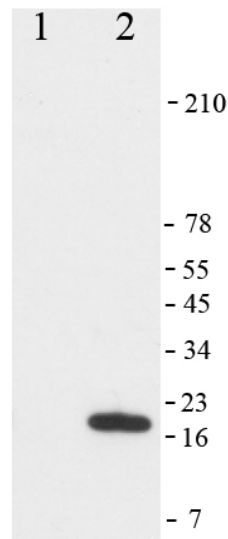
### Control

HeLa acid extracts

### Quality Control Testing

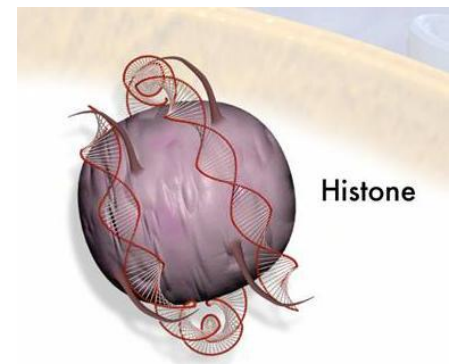
#### Western Blot Analysis:

1:2000–1:8000 dilution of this lot detected methylated histone H3 in acid extracted proteins from HeLa cells, but not recombinant unmethylated Histone H3 (Catalog # 14-494).



#### Western Blot Analysis

Recombinant unmethylated Histone H3 (Lane 1) and HeLa acid extracts (Lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-trimethyl Histone H3 (Lys 4) (1:4000). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates trimethyl-histone H3 (Lys4) (17 kDa).



#### References

1. Strahl, B.D., *et al.* (1999). Proc. Natl. Acad. Sci. USA. 96:14967-14972.
2. Fingerman, I.M., *et al.* (2005) J. Biol. Chem. 280:28761-28765.
3. Egelhofer, T.A., *et al.* (2011). Nat Struct Mol Biol. 18(1):91-93.
4. Easwaran, H., *et al.* (2012). Genome Res. 22: 837 - 849.
5. De, S., *et al.* (2011). Mol. Cell. Biol. 31: 1512 - 1527.

### Additional Research Applications

#### Beadlyte® Histone-Peptide Specificity Assay:

1:10,000-1:50,000 dilutions of a previous lot were incubated with histone H3 peptides containing various modifications conjugated to Luminex® microspheres. Slight crossreactivity with peptide containing dimethyl-lysine 4 was detected (see Figure B).

Chromatin Immunoprecipitation: Reported by an independent laboratory on a previous lot.

**APPLICATION LEGEND:** WB Western Blotting ChIP Chromatin Immunoprecipitation DB Dot Blot BD Beadlyte® Assay IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue) ChIP-Seq Chromatin Immunoprecipitation Sequence

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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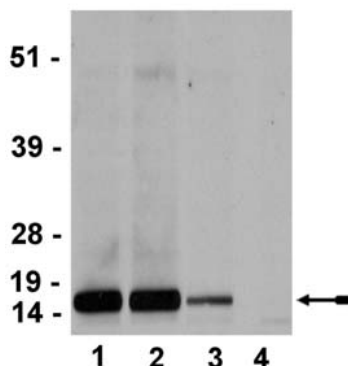
**Additional Research Applications:**Dot blot Analysis:

A representative lot of this antibody was used by an independent laboratory for DB (Bing Ren Laboratory, UC San Diego). See Egelhofer, T.A., *et al.* (2011).

ChIP-Seq Analysis:

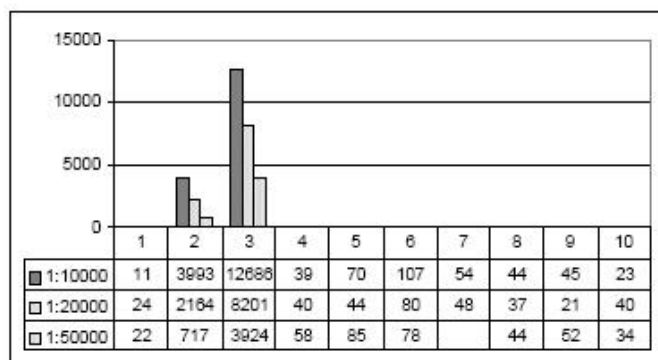
A representative lot of this antibody was used by an independent laboratory for ChIP-Seq. See Egelhofer, T.A., *et al.* (2011). See Easwaran, H., *et al.* (2012). See De, S., *et al.* (2011).

Peptide Inhibition: Specificity of a previous lot confirmed by the ability of 0.1  $\mu$ M of the immunizing peptide to completely abolish detection of histone H3 in immunoblot analysis of HeLa acid extracts (**Figure A**, Lane 4). Signal reduction was detected with preincubation of this lot with peptide containing dimethyl-lysine 4 (**Figure A**, Lane 3).



**Figure A**  
**Western Blot Analysis and Peptide Inhibition**

Representative blot from a previous lot. HeLa acid extracts were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-trimethyl Histone H3 (Lys 4) (0.07  $\mu$ g/mL, Lane 1) or preabsorbed with 0.1  $\mu$ M histone H3 peptides with the following modifications:  
Lane 2: monomethyl-lysine 4  
Lane 3: dimethyl-lysine 4  
Lane 4: trimethyl-lysine 4  
Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates trimethyl-histone H3 (Lys4) (17kDa).



**Figure B**  
**Beadlyte® Histone-Peptide Specificity Assay**

Representative data from a previous lot. 0.135-0.67 mg/mL of a previous lot were incubated with a cocktail of microspheres conjugated to histone H4 peptides with the following modifications:

1. monomethyl-lysine 4
2. dimethyl-lysine 4
3. trimethyl-lysine 4
4. trimethyl-lysine 9
5. trimethyl-lysine 23
6. trimethyl-lysine 27
7. trimethyl-lysine 36
8. trimethyl-lysine 79
9. dimethyl-lysine 14
10. unmodified, containing lysine 4

Unbound antibody was then removed by filtration. Bound antibody complexes were detected with a biotin-conjugated anti-rabbit secondary antibody followed by a phycoerythrin-streptavidin conjugate. Fluorescence was read on a Luminex® 100™ instrument.

Median Fluorescence Intensity (MFI) is plotted.

**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 3% nonfat dry milk (Catalog # 20-200) and 0.05% Tween 20 (TBST-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with 1:2000 – 1:8000 of anti-trimethyl-Histone H3 (Lys4), diluted in freshly prepared TBST-MLK 1 hour 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.
9. Use detection method of choice (enhanced chemiluminescence was used).

**Anti-trimethyl-Histone H3 (Lys4), clone MC315**Cat # 04-745  
Lot # 2462884**Acid Extraction of Proteins from HeLa Cells**

1. Scrape the cells from the plate.
2. Pellet the cells by centrifugation at 200 x g for 10 minutes.
3. Decant the supernatant fraction.
4. Suspend the cells with 10-15 volumes of PBS and centrifuge at 200 x g for 10 minutes.
5. Decant supernatant fraction (PBS wash).
6. Suspend the cell pellet in 5-10 volumes of lysis buffer.
7. Add hydrochloric acid to a final concentration of 0.2 M (0.2 N). Use polypropylene tubes.
8. Incubate on ice for 30 minutes.
9. Centrifuge at 11,000 x g for 10 minutes at 4°C.
10. Keep the supernatant fraction which contains the acid soluble proteins and discard the acid-insoluble pellet.
11. Dialyze the supernatant against 200 mL 0.1 M (0.1 N) acetic acid, twice for 1-2 hours each.
12. Dialyze three times against 200 mL H<sub>2</sub>O for 1 hour, 3 hours, and overnight, respectively. The protein can be quantified and lyophilized or stored at -70°C.

**Lysis buffer:**

10 mM HEPES, pH 7.9	*0.5 mM DTT
1.5 mM MgCl <sub>2</sub>	*1.5 mM PMSF
10 mM KCl	

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

Produced in collaboration with **EPITOMICS**

RELATED PRODUCTS (specific)		RELATED PRODUCTS (non-specific)	
cat #	description	cat #	description
05-809	■ Anti-trimethyl (Lys9)-phospho (Ser10)-Histone H3	IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
07-449	■ Anti-trimethyl-Histone H3 (Lys27)	IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
07-549	■ Anti-trimethyl-Histone H3 (Lys36)	IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
05-801	■ Anti-trimethyl-Histone H3 (Lys36), clone MC86	ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
07-473	■ Anti-trimethyl-Histone H3 (Lys4)	ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
05-745	■ Anti-trimethyl-Histone H3 (Lys4), clone MC315	IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
07-523	■ Anti-trimethyl-Histone H3 (Lys9)	WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
07-442	■ Anti-trimethyl-Histone H3 (Lys9)	17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
07-527	■ Anti-trimethyl-phospho (Lys9/Ser10 & Lys27/Ser28) Histone H3	2060	■ Re-Blot Western Blot Recycling Kit
05-788	■ Anti-trimethyl-phospho (Lys9/Ser10 & Lys27/Ser28) Histone H3, clone NL35	2500	■ Re-Blot Plus Western Blot Recycling Kit
17-622	■ ChIPAb+ Trimethyl-Histone H3 (Lys27)	B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
17-625	■ ChIPAb+ Trimethyl-Histone H3 (Lys9)		
12-565	■ Trimethyl-Histone H3 (Lys27) Peptide, biotin conjugate		
12-564	■ Trimethyl-Histone H3 (Lys4) Peptide, biotin conjugate		
12-568	■ Trimethyl-Histone H3 (Lys9) Peptide, biotin conjugate		
17-245	■ Acetyl-Histone H3 Immunoprecipitation (ChIP) Assay Kit		
12-348	■ Goat Anti-Rabbit IgG		

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