

ChIPAb+ Trimethyl-Histone H3 (Lys9)

Polyclonal Antibody/Primer Set

Cat. #17-625

Lot #DAM1591825

pack size: 25 assays

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Store at -20°C



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
ChIP, WB, MPLX	WR	IgG	Trimethyl Lys9	Rb	17 kDa	NM_003493

Background

All ChIPAb+ antibodies are individually validated for chromatin precipitation, every lot, every time. Each ChIPAb+ antibody set includes control primers (tested every lot by qPCR) to biologically validate your IP results in a locus-specific context. The qPCR protocol and primer sequences are provided, allowing researchers to validate ChIP protocols when using our antibody in their chromatin context. Each set also includes a negative control antibody to ensure specificity of the ChIP reaction.

The ChIPAb+ Trimethyl-Histone H3 (Lys9) set includes the Anti-trimethyl-Histone H3 (Lys9) antibody, a negative control antibody (purified rabbit IgG), and qPCR primers which amplify a 170 bp region within the promoter of the murine p16 gene. The trimethyl-histone H3 (Lys9) and negative control antibodies are supplied in a scalable "per ChIP" reaction size and can be used to functionally validate the precipitation of trimethyl-histone H3 (Lys9) associated chromatin.

Presentation

25 assays per set. Recommended use: ~4 µg antibody per chromatin immunoprecipitation (dependent upon biological context).

Components:

Anti-trimethyl-Histone H3 (Lys9) (rabbit polyclonal IgG), Cat.# 07-442. One vial containing 100 µg protein A purified IgG in 100 µL of 0.02 M Phosphate buffer, pH7.4, 0.25 M NaCl, 0.05% sodium azide with 30% glycerol. Store at -20°C.

Normal Rabbit IgG, Cat. #PP64B. One vial containing 125 µg of normal rabbit IgG in 125 µL storage buffer containing 0.1% sodium azide. Store at -20°C.

ChIP Primers, p16 (mouse) Cat. # CS200602. One vial containing 75 µL of 5 µM of each primer specific for mouse p16 promoter. Store at -20°C.

FOR: ACA CTC CTT GCC TAC CTG AA
REV: CGA ACT CGA GGA GAG CCA TC

Specificity

Recognizes histone H3, Mr 17 kDa, trimethylated at lysine 9.

Species Cross-reactivity

Human, mouse. The immunogen sequence is identical in a wide range of animal and plant species, so broad cross-reactivity is expected.

Immunogen

The trimethyl-histone H3 (Lys9) purified antibody is made against BSA-conjugated, synthetic peptide containing the sequence ...AR[me3K]S... in which me3K corresponds to trimethyl lysine 9 of human Histone H3.

Molecular Weight

Trimethyl-histone H3 at ~17 kDa

Storage and Handling

Stable for 1 year at -20°C from date of receipt. Aliquot upon thawing, avoid freeze thaw cycles.

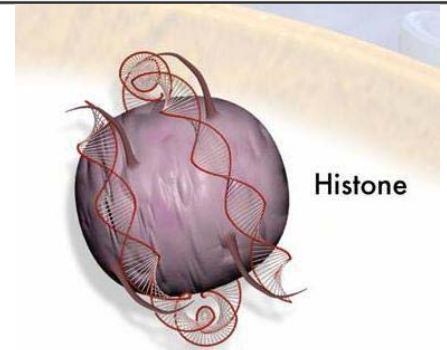
Control

Included negative control rabbit IgG antibody and control primers specific for murine p16 promoter.

Quality Control Testing

Chromatin Immunoprecipitation:

Sonicated Chromatin prepared from 3x10⁶ NIH3T3 L1 cells were subjected to chromatin immunoprecipitation using 4 µg of either normal rabbit IgG, (Cat. #PP64B), or Anti-trimethyl-Histone H3 (Lys9) antibody (Cat. #CS200604) and the Magna ChIP A kit (Cat.



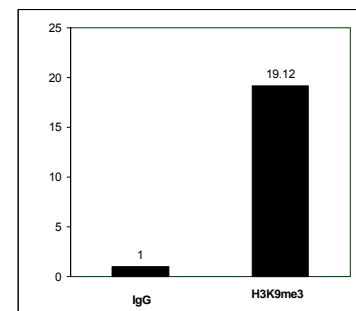
References

1. Rea, S., et al., (2000). *Nature* **406**:593-599.
2. Jenuwein, T., et al., (2002). *Curr. Opin. Cell Biol.* **14**:286-298.

#17-610). Successful enrichment of trimethyl-histone H3 (Lys9)-associated DNA fragments was verified by qPCR using ChIP Primers mouse p16 (Cat.# CS200602) flanking the mouse p16 promoter (Figure 1).

Please refer to the EZ-Magna A ChIP™ (Cat. #17-408) or EZ-ChIP™ (Cat. #17-371) protocol for experimental details.

Figure 1:



APPLICATION LEGEND: WB Western Blotting ChIP Chromatin Immunoprecipitation MPLX Multiplex

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

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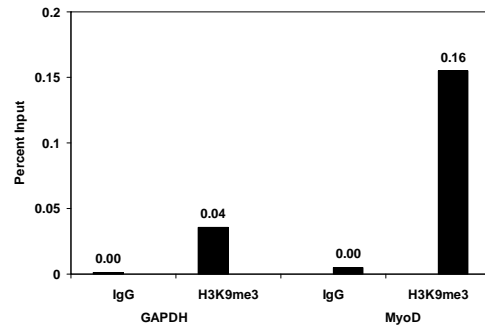
Additional Research Applications

Chromatin Immunoprecipitation:

Sonicated chromatin prepared from 2x10⁶ HeLa cells were subjected to chromatin immunoprecipitation using 4 µg purified antibody or normal rabbit IgG and the Magna ChIP™ A kit (Cat. #17-610). Successful enrichment of trimethyl-Histone H3 (Lys9) associated DNA fragments was verified by qPCR using ChIP Primers GAPDH (Cat. #22-004) flanking the human GAPDH promoter and primers targeting the promoter of human MyoD.

Please refer to the EZ-Magna ChIP™ A (Cat. # 17-408) or EZ-ChIP™ (Cat. # 17-371) kit protocols for experimental details.

Figure 2:

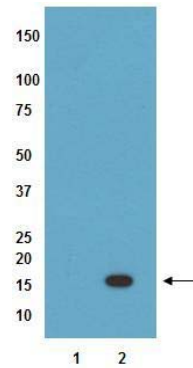


Western Blot Analysis:

Recombinant Histone H3 (Catalog # 14-494, Lane 1) and HeLa acid extract (Lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-trimethyl-Histone H3 (Lys9), (1 µg/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates trimethyl-Histone H3 (Lys9) (~17 kDa).

Figure 3:



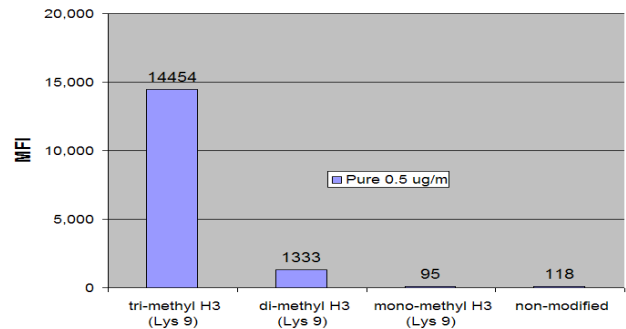
Beadlyte® Histone Peptide Specificity Assay:

0.5 µg/ml of purified anti-dimethyl-Histone H3 (Lys9) was incubated with a cocktail of microspheres conjugated to histone H3 peptides with the following modifications:

1. trimethyl-lysine 9
2. dimethyl-lysine 9
3. monomethyl-lysine 9
4. Unmodified H3

Unbound antibody was then removed by filtration. Peptide antibody complexes were incubated with a PE-conjugated anti-rabbit secondary antibody. Fluorescence was read on a Luminex® 100™ instrument. Median Fluorescence intensity (MFI) is plotted.

Figure 4:



PROTOCOL

qPCR Protocol

qPCR run parameters and reagent setup are listed below. Preparing qPCR master mix is recommended for multiple PCR reactions. We suggest performing triplicate for qPCR of each individual ChIP reaction.

qPCR reagent assembly for 1 reaction:

ChIP final product	2.0 µL
ddH ₂ O	9.5 µL
SYBR-Green Master Mix	12.5 µL
Primer mix	1.0 µL
Total	25 µL

qPCR parameters:

Initial Denaturation: 94°C 10 min
 Denature: 94°C 20 sec
 Anneal and Extension: 60°C 1 min } 50 times

Western Blotting Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate or tissue sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na₃VO₄; 1 mM NaF) and transfer the proteins to PVDF (Immobilon-P). Wash the blotted PVDF (Immobilon-P) twice with Tris/0.05% Tween® (TBST).
2. Block the blotted PVDF in freshly prepared TBST containing 5% nonfat dry milk (Catalog #20-200), for 30 minutes at room temperature with constant agitation.
3. Incubate the PVDF with 1 µg/mL anti-trimethyl-Histone H3 (Lys9), diluted in freshly prepared TBST-MILK at room temperature for 2 hours (or overnight at 4°C) with agitation.
4. Wash the PVDF 3 times with TBST for 3-5 minutes each.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG (H+L), Catalog #AP132P was used) in TBST-MILK for 60 minutes at room temperature with agitation.
6. Wash the PVDF 3 times in TBST for 3-5 minutes each wash. Use detection method of choice (enhanced chemiluminescence was used).

RELATED PRODUCTS (specific)

CAT #	DESCRIPTION
17-608	■ ChIPAb+ HDAC1
17-613	■ ChIPAb+ p53
17-601	■ ChIPAb+ Sp1
17-622	■ ChIPAb+ Trimethyl-Histone H3 (Lys27)
17-648	■ ChIPAb+ Dimethyl-Histone H3 (Lys9)
17-603	■ ChIPAb+ Estrogen Receptor α
17-604	■ ChIPAb+ LEF1
17-615	■ ChIPAb+ Acetyl-Histone H3
17-620	■ ChIPAb+ RNA Polymerase II
17-630	■ ChIPAb+ Acetyl-Histone H4
17-658	■ ChIPAb+ Acetyl-Histone H3 (Lys9) Purified
17-614	■ ChIPAb+ Trimethyl-Histone H3 (Lys4)
17-643	■ ChIPAb+ Monomethyl-Histone H3 (Lys27)
17-611	■ Magna ChIP™ G Chromatin Immunoprecipitation Kit
17-409	■ EZ-Magna ChIP™ G Chromatin Immunoprecipitation Kit
17-610	■ Magna ChIP™ A Chromatin Immunoprecipitation Kit
17-408	■ EZ-Magna ChIP™ A Chromatin Immunoprecipitation Kit
17-371	■ EZ-ChIP™
17-295	■ Chromatin Immunoprecipitation (ChIP) Assay Kit

RELATED PRODUCTS (non-specific)

CAT #	DESCRIPTION
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

