

Certificate of Analysis

ChIPAb+ Trimethyl-Histone H3 (Lys9)

Catalog # 17-625

Lot # DAM1437101

Product Description: Every lot of the ChIPAb+ line of antibodies is individually validated for chromatin precipitation, in order to guarantee successful ChIP assays every time. Each antibody includes a control primer set for performance confirmation. Trimethyl-Histone H3 (Lys9) antibody is functionally validated in the precipitation of chromatin that carries trimethyl-Histone H3 (Lys9).

The qPCR primers included amplifies the mouse p16 promoter region where trimethyl-Histone H3 (Lys9) is enriched.

Quantity: 25 assays per kit, ~4 μL per chromatin immunoprecipitation.
(Dependent upon biological context)

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Kit Components

Anti-trimethyl-Histone H3 (Lys9) (rabbit crude serum), Cat.# CS200604. 1 vial containing 100 μL serum. Store at -20°C . The 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. It recognizes human and mouse trimethyl-Histone H3 (Lys9). Broad species cross-reactivity expected.

ChIP primers p16 mouse, Cat.# CS200602. 1 vial containing 75 μL of 5 μM of each control 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

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

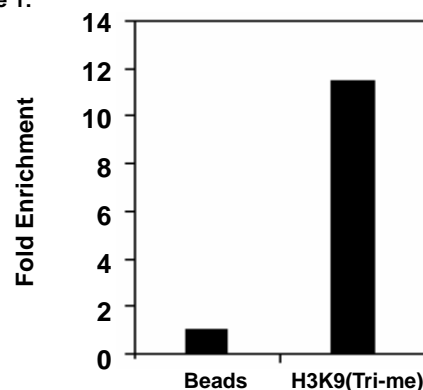
Quality Control Testing

ChIP Analysis:

Sonicated Chromatin prepared from 3×10^6 NIH3T3 L1 cells were subjected to chromatin immunoprecipitation using 4 μL serum or magnetic protein A beads only and the Magna ChIP™ A kit (Cat.# 17-610) Rapid Protocol (2 hour IP). Successful enrichment of trimethyl-Histone H3 (Lys9) associated DNA fragments was verified by qPCR using ChIP Primers p16 mouse (Cat.# CS200602) flanking the mouse p16 promoter (Figure 1).

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

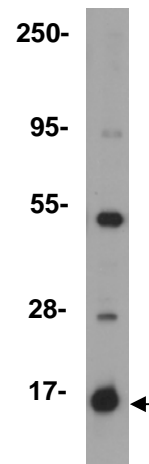
Figure 1:



Immunoblot Analysis:

Acid-extracted histones from HeLa cells were resolved by electrophoresis, transferred to nitrocellulose and probed with anti trimethyl-Histone H3 (Lys9) (1:1000 dilution). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system (Figure 2). Arrow indicates trimethyl-Histone H3 (Lys9).

Figure 2:



qPCR Protocol

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

qPCR reagent assembly for 1 reaction:

ChIP final product	5 μ L
ddH ₂ O	6.5 μ L
Sybr-Green Master Mix	12.5 μ L
Primer mix	1 μ L
<hr/> Total	<hr/> 25 μ L

qPCR parameters:

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

References:

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

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