

Certificate of Analysis

ChIPAb+ Trimethyl-Histone H3 (Lys27)
Catalog # 17-622
Lot # DAM1561713

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 (Lys27) antibody is functionally validated in the precipitation of chromatin that carries trimethyl-Histone H3 (Lys27). The qPCR primers included amplifies the mouse p16 promoter region where trimethyl-Histone H3 (Lys27) is enriched.

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

Storage and Stability: Stable for 1 year at -20°C from date of shipment.

Kit Components

Anti-trimethyl-Histone H3 (Lys27) (rabbit crude serum), Cat.# CS200603. 1 vial containing 100 µL serum. Store at -20°C. The antibody is made against KLH-conjugated, synthetic 2X-branched peptide containing the sequence ...AR(me3K)SAP... in which me3K corresponds to trimethyl-lysine at residue 27 of human Histone H3. It recognizes human and mouse trimethyl-Histone H3 (Lys27). 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

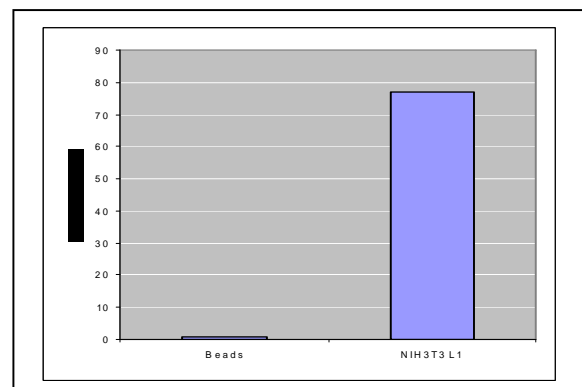
Quality Control Testing

ChIP Analysis:

Sonicated Chromatin prepared from 3x10⁶ 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). Successful enrichment of trimethyl-Histone H3 (Lys27) associated DNA fragments was verified by qPCR using ChIP Primers mouse p16 (Cat.# CS200602) flanking the mouse p16 promoter.

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

Figure 1:

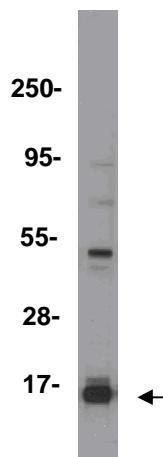


Additional Application Testing

Immunoblot Analysis:

Acid-extracted histones from HeLa cells was resolved by electrophoresis, transferred to nitrocellulose and probed with anti trimethyl-Histone H3 (Lys27) (1:500 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 (Lys27).

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 | 2 μ L |
| ddH ₂ O | 9.5 μ L |
| Sybr-Green Master Mix | 12.5 μ L |
| Primer mix | 1 μ L |
| <hr/> | |
| Total | 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. Kohlmaier, A., *et al* *PLoS Biol* **2**: E171. 2004.
2. Perez-Burgos, L., *et al* *Methods Enzymol* **376**: 234-54. 2004
3. Peters, A. H., *et al* *Mol Cell* **12**: 1577-89. 2003

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