

## Certificate of Analysis

### ChIPAb+ Trimethyl-Histone H3 (Lys27)

Catalog # 17-622

Lot # DAM1514145

**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.

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

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

### 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**

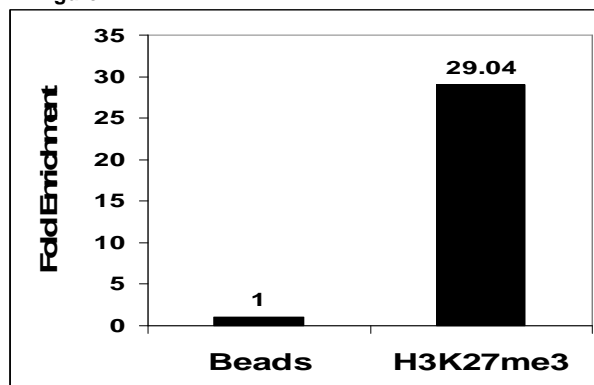
### Quality Control Testing

#### ChIP Analysis:

Sonicated Chromatin prepared from 3x10<sup>6</sup> 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:

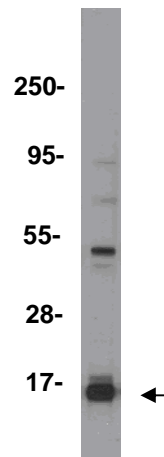


### Additional Application Testing

#### 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:



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### 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 $\mu$ L
ddH <sub>2</sub> O	9.5 $\mu$ L
Sybr-Green Master Mix	12.5 $\mu$ L
Primer mix	1 $\mu$ L
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Total	25 $\mu$ L

#### qPCR parameters:

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

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#### 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