

## Certificate of Analysis

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

Catalog # 17-622

Lot # DAM1478803

**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**

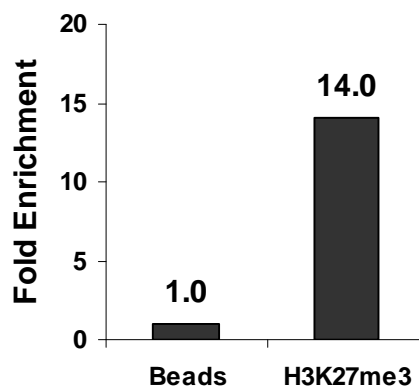
### Quality Control Testing

#### ChIP Analysis:

Sonicated Chromatin prepared from  $3 \times 10^6$  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). 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:

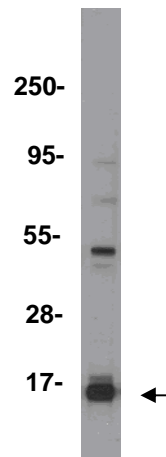


**Additional Application Testing**

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

**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