

# ChIPAb+™ HDAC1

Monoclonal Antibody/Primer Set

Cat. # 17-10199

Lot # 2053033

pack size: 25 assays

FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES  
NOT FOR HUMAN OR ANIMAL CONSUMPTION

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, IF	H, (M, R, Ca, Ht)	N/A	a.a. 467-482	Rb	~62 kDa	NP_032254

### 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+ HDAC1 polyclonal set includes the HDAC1 antibody, a Normal Rabbit IgG, and control primers which amplify a 88 bp region of ChIP Primers p21. The HDAC1 and negative controls are supplied in a scalable "per ChIP" reaction size and can be used to functionally validate the precipitation of HDAC1-associated chromatin.

### Presentation

25 assays per set. Recommended use: ~2 µg of antibody per chromatin immunoprecipitation (dependent upon biological context).

### Components:

**Anti-HDAC1** (rabbit polyclonal), Part No. CS207297. One vial containing 50 µg of Anti-HDAC1 IgG in PBS with 1 mg/mL BSA and ≤0.1% sodium azide before the addition of glycerol to 30%. Store at -20°C.

**Concentration:** 0.7 mg/mL

**Normal Rabbit IgG**, Part No. PP64B. One vial containing 125 µg of rabbit IgG in 125 µL of storage buffer containing 0.05% sodium azide. Store at -20°C.

**ChIP Primers p21**, Part No. CS200575. One vial containing 75 µL of 5 µM of each primer specific for a region of the human p21 (WAF1/CIP1/CDKN1A) promoter. Store at -20°C.

FOR: CCC ACA GCA GAG GAG AAA GAA

REV: CTG GAA ATC TCT GCC CAG ACA

### Specificity

This antibody recognizes amino acids 467-482 of HDAC1.

### Species Cross-reactivity

Demonstrated to react with human. Predicted to react with rat, mouse, canine, and hamster based on 100% sequence homology.

### Immunogen

Synthetic peptide corresponding to amino acids 467-482 of human HDAC1.

### Molecular Weight

~62 kDa observed.

### Storage and Handling

Stable for 1 year at -20°C from date of receipt. Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

### Controls

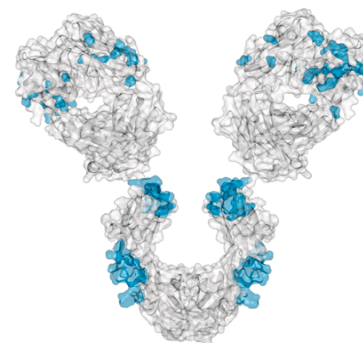
Includes normal rabbit IgG and primers specific for human p21.

### Quality Control Testing

#### Chromatin Immunoprecipitation:

Representative lot data.

Sonicated chromatin prepared from U2OS cells (3 X 10E6 cell equivalents per IP) were subjected to chromatin immunoprecipitation using 2 µg of either Normal Rabbit IgG, (Part No. PP64B), or 2 µg of Anti-HDAC1 (Part No. CS207297) and the Magna ChIP™ A Kit



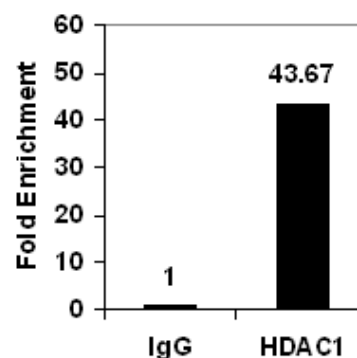
### References

1. Lagger, G., et al., (2003). *Mol. Cell. Biol.* 23:2669-2679.
2. Gaughan, L., et al., (2002). *J. Biol. Chem.* 277:25904-25913.
3. Kaeser M.D., et al., (2002). *Proc Natl Acad Sci.* 99:95-100.
4. Doetzihofer, A. et al., (1999). *Mol. Cell. Biol.* 19: 5504-5511.

(Cat. # 17-610). Successful immunoprecipitation of HDAC1 associated DNA fragments was verified by qPCR using ChIP Primers, p21 (Part No. CS200575) flanking an Sp1 binding site in the human p21 promoter (**Figure 1**).

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

Figure 1:



**APPLICATION LEGEND:** WB Western Blotting ChIP Chromatin Immunoprecipitation IP Immunoprecipitation IC Immunocytometry BD Beadlyte® Assay DB Dot Blot

**SPECIES LEGEND:** H Human Xn Xenopus M Mouse R Rat Vrt Vertebrates ( ) Predicted Reactivity

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## Additional Research Applications

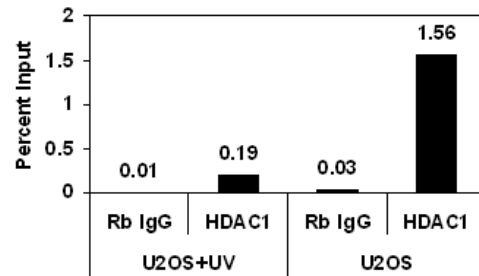
### Chromatin Immunoprecipitation:

Representative lot data.

Sonicated chromatin prepared from untreated or UV treated (6 hrs, 50 joules/m<sup>2</sup>.) U2OS cells (3 X 10<sup>6</sup> cell equivalents per IP) was subjected to chromatin immunoprecipitation using using 2 µg of either Normal Rabbit IgG, (Part No. PP64B), or 2 µg of Anti-HDAC1 (Part No. CS207297) and the Magna ChIP™ A Kit (Cat. # 17-610). Successful immunoprecipitation of HDAC1 associated DNA fragments was verified by qPCR using ChIP Primers, p21 (Part No. CS200575). (Figure 2). Data is presented as percent input of each IP sample relative to input chromatin for each amplicon and ChIP sample as indicated.

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

Figure 2:



### Western Blot Analysis:

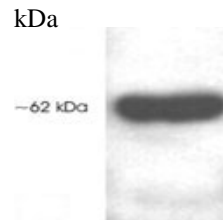
Representative lot data.

0.5 µg/mL from a representative lot detected HDAC1 in HeLa cells. (Figure 3).

### Immunofluorescence Analysis:

2 µg/mL from a representative lot detected HDAC1 in HeLa cells.

Figure 3:



## 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 <sub>2</sub> O	9.5 µL
SYBR-Green Master Mix	12.5 µL
Primer mix	1.0 µL
<b>Total</b>	<b>25 µL</b>

#### qPCR parameters:

Initial Denaturation: 94°C 10 min

Denature: 94°C 20 sec

Anneal and Extension: 60°C 1 min

} 50 times

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**RELATED PRODUCTS (specific)**

CAT #	DESCRIPTION
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
17-608	■ ChIPAb+ HDAC1
07-1575	■ Anti-phospho HDAC1 (Ser421/Ser423)

**RELATED PRODUCTS (non-specific)**

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

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