ChIPAb+ Trimethyl Histone H3 (Lys36)

Polyclonal Antibody

Cat. # 17-10493

Lot # 3059387

Pack size: 25 Assays

Store at -20°C

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

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, ChIP, ICC, DB	H, M	N/A	Methylated Lys36	Rb	~17 kDa	NP_003520

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+™ Trimethyl Histone H3 (Lys36) set includes a Trimethyl Histone H3 (Lys36) antibody, a Normal Rabbit IgG, and positive control primers which amplify a 87 bp region of the human GAPDH coding region. The Trimethyl Histone H3 (Lys36) antibody and negative control IgG are supplied in a scalable "per ChIP" reaction size and can be used to functionally validate the precipitation of trimethyl Histone H3 (Lys36) associated chromatin.

Presentation

25 assays per set. Recommended use: 0.1 µg of antibody per chromatin immunoprecipitation (dependent upon biological context).

Components:

Anti-Trimethyl Histone H3 (Lys36) (Rabbit Polyclonal), Part No. CS207341. One vial containing 50 µL (0.05 mg/mL) purified rabbit polyclonal in buffer containing 0.1 M Tris-Glycine (pH7.4),150mM NaCl, and 0.05% sodium azide, before the addition of 30% glycerol. Store at -20°C.

Normal Rabbit IgG, Part No. CS200581. One vial containing 7.5 µg Rabbit IgG in 75 µL storage buffer containing 0.05% sodium azide. Store at -20°C.

ChIP Primers, GAPDH coding D2. Part No. CS207323. One vial containing 75 µL of 5 µM of each primer specific for human GAPDH coding region (chr12:6647453+6647539 hg19 build) . Store at -20°C.

FOR: 5' GCC ATG TAG ACC CCT TGA AGA G 3'

REV: 5' ACT GGT TGA GCA CAG GGT ACT TTA T 3'

Species Cross-reactivity

Demonstrated to react with Human and Mouse.

Immunogen

Linear peptide corresponding to human Histone H3 trimethylated at Lys36 with peptide sequence APATGGV(K)KPHRYRPGC

Molecular Weight

~17 kDa

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.

Controls

Includes normal rabbit IgG and primers specific for human GAPDH coding region.

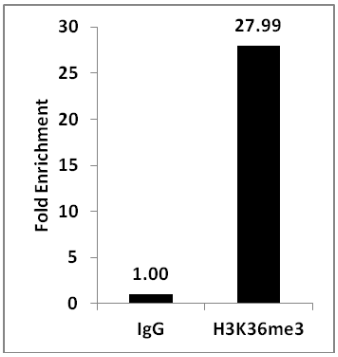
Quality Control Testing

Chromatin Immunoprecipitation:

Sonicated chromatin prepared from HeLa cells (1e5 cell equivalents per IP) were subjected to chromatin immunoprecipitation using 0.1 µg of either Normal Rabbit IgG (Part No. CS200581), or 0.1 µg Anti-Trimethyl-Histone H3 (Lys36) (Part No.CS207341) and the Magna ChIP™ HiSens Kit (Cat. # 17-10460). Successful immunoprecipitation of trimethyl-Histone H3 (Lys36) associated DNA fragments was verified by qPCR using ChIP Primers, GAPDH coding D2 (Part No. CS207323) (Figure 1).

Please refer to the Magna ChIP™ HiSens (Cat. # 17-10460) or EZ-Magna ChIP™ HiSens (Cat. # 17-10461) protocol for experimental details.

Figure 1:



References

1. Bannister, A. *et al*. (2005) *J Biol Chem* 280(18):17732-6.
2. Lin, C.H., *et al*. (2008). *Mol Cell*. 32(5):696-706.
3. Shi, X., *et al*. (2007). *J. Biol. Chem*. 282(4): 2450-2455.
4. Rando, O.J. (2007). *Curr. Opin. Genet. Dev*. 17(2):94-99.

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Additional Research Applications

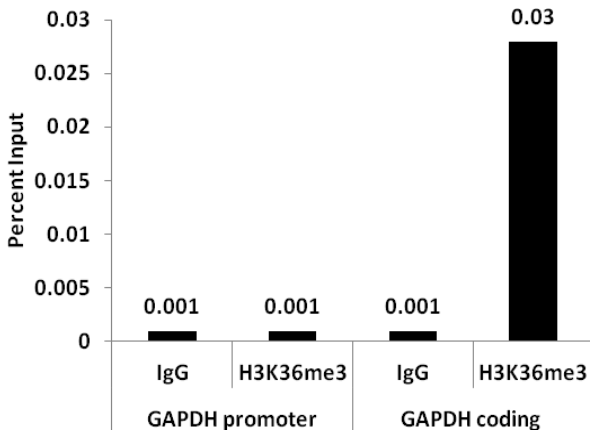
Chromatin Immunoprecipitation:

Sonicated chromatin prepared from HeLa cells (1e5 cell equivalents per IP) were subjected to chromatin immunoprecipitation using 0.1 µg of either Normal Rabbit IgG (Part No. CS200581), or 0.1 µg Anti-Trimethyl-Histone H3 (Lys36) (Part No.CS207341) and the Magna ChIP™ HiSens (Cat. # 17-10460). Successful immunoprecipitation of trimethyl-Histone H3 (Lys36) associated DNA fragments was verified by qPCR using ChIP Primers, GAPDH coding D2 (Part No. CS207323) as a positive locus, and GAPDH promoter (Part No. 22-004) as a negative locus. (Figure 2). Data are presented as percent input of each IP sample relative to input chromatin for each amplicon and ChIP sample as indicated.

Please refer to the Magna ChIP™ HiSens (Cat. # 17-10460) or EZ-MagnaChIP™ HiSens(Cat. # 17-10461) protocol for experimental details.

Chromatin Immunoprecipitation:
GAPDH promoter primer sequences (chr12:6643539+6643704, hg19 build):
F= TAC TAG CGG TTT TAC GGG CG
R=TCG AAC AGG AGG AGC AGA GAG CGA

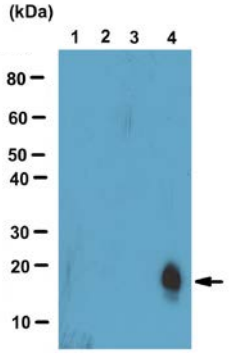
Figure 2:



Western Blotting Analysis:

Western Blot Analysis: A 1:2000 dilution of this antibody detected Histone on 10 µg of Histone H3 recombinant proteins. (Figure 3)

Figure 3:



Western Blotting Analysis:
Representative lot data
Recombinant Histone H3 (lane 1), Monomethyl Histone H3 (Lys36) (lane 2), Dimethyl Histone H3 (Lys36) (lane 3), and Trimethyl Histone H3 (Lys36) (lane 4) proteins were probed with Anti-trimethyl Histone H3 (Lys36) (1:2,000 dilution). Proteins were visualized using a Donkey Anti-Rabbit IgG seconday antibody conjugated to HRP and a chemiluminescence detection system.

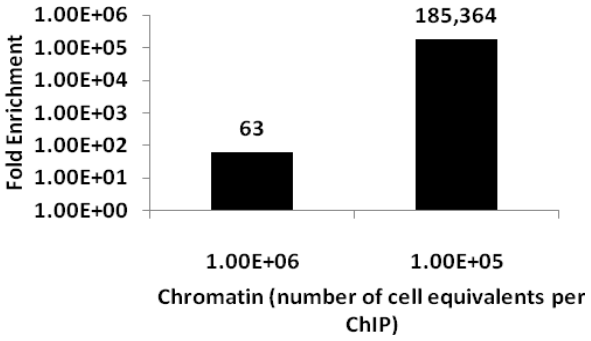
Arrow indicates Histone H3 (~17 kDa).

Chromatin Immunoprecipitation:

Sonicated chromatin prepared from HeLa cells (1e5 and 1e6 cell equivalents per IP) were subjected to chromatin immunoprecipitation using 0.1 µg of either Normal Rabbit IgG (Part No. CS200581), or 0.1 µg Anti-Trimethyl-Histone H3 (Lys36) (Part No.CS207341) and the Magna ChIP™ HiSens Kit (Cat. # 17-10460). Successful immunoprecipitation of trimethyl Histone H3 (Lys36) associated DNA fragments was verified by qPCR using ChIP Primers, GAPDH coding D2 (Part No. CS207323). Figure 4 shows enrichment over IgG.

Please refer to the Magna ChIP™ HiSens (Cat. # 17-10460) or EZ-Magna ChIP™ HiSens (Cat. # 17-10461) protocol for experimental details.

Figure 4:



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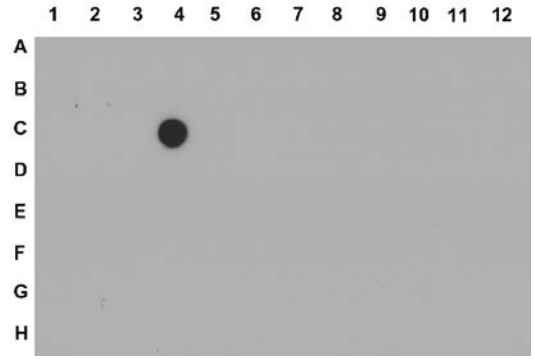
Immunofluorescence Analysis: A representative lot was used by an independent laboratory in NIH/3T3 fibroblasts. (Laboratory of Dr. Rob Klose, Oxford University)
Dot Blot Analysis: A 1:500 dilution from a representative lot detected trimethyl Histone H3 (Lys36).

Figure 5:



Dot Blot Specificity Analysis:
Representative lot data.
Histone peptides with various modifications (see table) were transferred to PVDF membrane and probed with Anti-trimethyl Histone H3 (Lys36) (1:500 dilution). Proteins were visualized using a Donkey Anti-Rabbit IgG secondary antibody conjugated to HRP and a chemiluminescence detection system.

	1	2	3	4	5	6	7	8	9	10	11	12
A: 40 ng B: 4 ng	unmod Histone H3(Lys4)	monomethyl-Histone H3(Lys4)	dimethyl-Histone H3(Lys4)	trimethyl-Histone H3(Lys4)	unmod Histone H3(Lys9)	monomethyl-Histone H3 (Lys9)	dimethyl-Histone H3 (Lys9)	trimethyl-Histone H3 (Lys9)	unmod. H3 (Lys27)	monomethyl-Histone H3 (Lys27)	dimethyl-Histone H3 (Lys27)	trimethyl-Histone H3 (Lys27)
C: 40 ng D: 4 ng	unmod H3 (Lys36)	monomethyl-H3 (Lys36)	dimethyl- H3 (Lys36)	trimethyl- H3 (Lys36)	unmod H3 (Lys37)	monomethyl-H3 (Lys37)	dimethyl- H3 (Lys37)	trimethyl- H3 (Lys37)	unmod. Histone H3 (Lys56)	monomethyl Histone H3 (Lys56)	unmod H3 (Lys79)	monomethyl-H3 (Lys79)
E: 40 ng F: 4 ng	dimethyl- H3 (Lys79)	trimethyl- H3 (Lys79)	unmod Histone H4 (Lys20)	monomethyl Histone H4 (Lys20)	dimethyl Histone H4 (Lys20)	trimethyl Histone H4 (Lys20)	unmod. H1.0 (Lys26)	monomethyl H1.0 (Lys26)	dimethyl H1.0 (Lys26)	trimethyl H1.0 (Lys26)	unmod. H2A (Lys127)	monomethyl H2A (Lys127)
G: 40 ng H: 4 ng	dimethyl H2A (Lys127)	trimethyl H2A (Lys127)	unmod. H2A (Lys118/119)	monomethyl-H2A (Lys118)	monomethyl-H2A (Lys119)	H2A (methyl-Lys118, ubiquityl-Lys119)	H2A (ubiquityl-Lys118, methyl-Lys119)	unmod. H2A (Lys17)	monomethy H2A (Lys17)	dimethy H2A (Lys17)	-	-

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