Anti-Trimethyl-Histone H3 (Lys27)

Polyclonal Antibody

Cat. # 07-449

Lot # 2475696

pack size: 200 µg

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, IP, ICC, DB, ChIP, ChIP-Seq	H, M, Ce	IgG	N/A	Rb	~17 kDa	NP_003493

Background

Histone H3 is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N-terminal tail, H3 is involved with the structure of the nucleosomes of the 'beads on a string' structure. The N-terminal tail of histone H3 protrudes from the globular nucleosome core and can undergo several different types of epigenetic modifications that influence cellular processes. These modifications include the covalent attachment of methyl or acetyl groups to lysine and arginine amino acids and the phosphorylation of serine or threonine.

Presentation

Purified rabbit IgG in buffer containing 0.1 M Tris-Glycine, pH 7.4, 0.15 M NaCl with 0.05% sodium azide and 30% glycerol.

Concentration

1 mg/mL

Epitope

N-terminus

Specificity

This antibody recognizes the N-terminus of Histone H3 trimethylated at Lys27. Broad species cross-reactivity is expected.

Immunogen

KLH-conjugated, synthetic 2X-branched peptide containing the sequence …AR(me_3K)SAP… in which me_3K corresponds to trimethyl-lysine at residue 27 of human histone H3.

Molecular Weight

~17 kDa

Method of Purification

Protein A purified

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20ºC. 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.

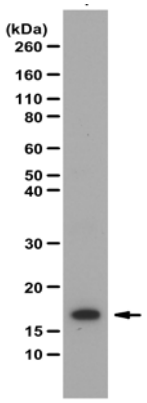
Control

HeLa acid extract

Quality Control Testing

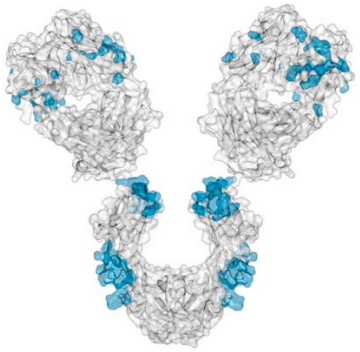
Evaluated by Western Blotting in HeLa acid extract.

Western Blotting Analysis: 0.05 µg/mL of this antibody detected Trimethyl-Histone H3 in 10 µg of HeLa acid extract.



Western Blotting Analysis
Representative lot data.
HeLa acid extract was probed with Cat. No. 07-449, Anti-Trimethyl-Histone H3 (Lys27) (0.05 µg/mL).
Proteins were visualized using a Goat Anti-Rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Trimethyl-Histone H3 (Lys27) (~17 kDa).



References

1. Kohlmaier, Alexander, *et al.* (2004). *PLoS Biol.* 2: E171.
2. Perez-Burgos, L., *et al.* (2004). *Methods Enzymol.* 376: 234-54.
3. Peters, A. H., *et al.* (2003). *Mol Cell.* 12: 1577-89.
4. Acevedo, Luis G., *et al.* (2007). *Bio Techniques.* 43: 791-7.
5. Egelhofer, T.A., *et al.* (2011). *Nat Struct Mol Biol*. 18(1):91-93.
6. Wang, H., *et al.* (2011). *PNAS.* 108: 14908 - 14913.
7. Neff, T., *et al.* (2012). *PNAS*. doi: 10.1073/pnas.

Additional Research Applications

Western Blotting Analysis: A representative lot was used by an independent laboratory in WB (Strome Lab, UC Santa Cruz). See Egelhofer, T.A., *et al*. (2011).

ChIP-Seq Analysis: A representative lot of this antibody was used by two independent laboratories in ChIP-Seq (Bing Ren Laboratory, UC San Diego and Strome Lab, UC Santa Cruz). See Egelhofer, T.A., *et al*. (2011). See Wang, H., *et al.* (2011). See Neff, T., *et al.* (2012).

APPLICATION LEGEND: **WB** Western Blotting **IP** Immunoprecipitation **ICC** Immunocytochemistry **DB** Dot Blot **IF** Immunofluorescence **ChIP** Chromatin Immunoprecipitation **ChIP-Seq** Chromatin Immunoprecipitation Sequence
SPECIES LEGEND: **H** Human **M** Mouse **R** Rat **Rb** Rabbit **Ce** *C. elegans* **( )** Predicted Reactivity

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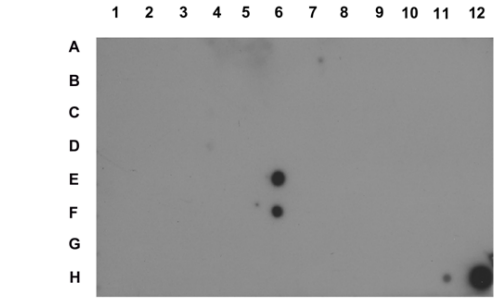
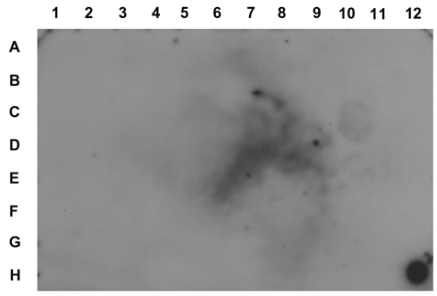
Additional Research Applications

Dot Blot (Specificity) Analysis: 1 µg/mL of this antibody detected Trimethyl-Histone H3 (Lys27), but not unmethylated Histone H3 (Lys27) or other peptides corresponding to modified histones in an Absurance™ Histone H3 Antibody Specificity Array (Cat. No. 16-667) and in an Absurance™ Histone H2A, H2B, H4 Antibody Specificity Array (Cat. No. 16-665).



	1	2	3	4	5	6	7	8	9	10	11	12	
A	H3 1-19 unmod	H3 1-19 R2me1	H3 1-19 R2me2a	H3 1-19 R2me2s	H3 1-19 T3P	H3 1-19 K4ac	H3 1-19 K4me1	H3 1-19 K4me2	H3 1-19 K4me3	H3 1-19 R8me1	H3 1-19 R8me2a	H3 1-19 R8me2s	100 ng
B	H3 1-19 unmod	H3 1-19 R2me1	H3 1-19 R2me2a	H3 1-19 R2me2s	H3 1-19 T3P	H3 1-19 K4ac	H3 1-19 K4me1	H3 1-19 K4me2	H3 1-19 K4me3	H3 1-19 R8me1	H3 1-19 R8me2a	H3 1-19 R8me2s	10 ng
C	H3 1-19 K9ac	H3 1-19 K9me1	H3 1-19 K9me2	H3 1-19 K9me3	H3 1-19 S10P	H3 1-19 T11P	H3 7-26 unmod	H3 7-26 K14ac	H3 7-26 R17me1	H3 7-26 R17me2a	H3 7-26 R17me2s	H3 7-26 K18ac	100 ng
D	H3 1-19 K9ac	H3 1-19 K9me1	H3 1-19 K9me2	H3 1-19 K9me3	H3 1-19 S10P	H3 1-19 T11P	H3 7-26 unmod	H3 7-26 K14ac	H3 7-26 R17me1	H3 7-26 R17me2a	H3 7-26 R17me2s	H3 7-26 K18ac	10 ng
E	H3 16-34 unmod	H3 16-34 K23ac	H3 16-34 K27ac	H3 16-34 K27me1	H3 16-34 K27me2	H3 16-34 K27me3	H3 16-34 R26me1	H3 16-34 R26me2a	H3 16-34 R26me2s	H3 16-34 S28P	H3 26-44 unmod	H3 26-44 K36ac	100 ng
F	H3 16-34 unmod	H3 16-34 K23ac	H3 16-34 K27ac	H3 16-34 K27me1	H3 16-34 K27me2	H3 16-34 K27me3	H3 16-34 R26me1	H3 16-34 R26me2a	H3 16-34 R26me2s	H3 16-34 S28P	H3 26-44 unmod	H3 26-44 K36ac	10 ng
G	H3 26-44 K36me1	H3 26-44 K36me2	H3 26-44 K36me3	H3 26-44 Y41P	H3 47-65 unmod	H3 47-65 K56ac	H3 71-89 unmod	H3 71-89 K79me1	H3 71-89 K79me2	H3 71-89 K79me3	Rat IgG 10 ng	Sheep IgG 10 ng	100 ng
H	H3 26-44 K36me1	H3 26-44 K36me2	H3 26-44 K36me3	H3 26-44 Y41P	H3 47-65 unmod	H3 47-65 K56ac	H3 71-89 unmod	H3 71-89 K79me1	H3 71-89 K79me2	H3 71-89 K79me3	Mouse IgG 10 ng	Rabbit IgG 10 ng	10 ng

Fig. 1



	1	2	3	4	5	6	7	8	9	10	11	12	
A	H2A 1-19 unmod	H2A 1-19 S1P	H2A 1-19 K5ac	H2A 1-19 K9ac	H2A 1-19 K13ac	H2A 110-129 unmod	H2A 110-129 T120P	H2A.X 124-142 unmod	H2A.X 124-142 S139P	H2A.X 124-142 Y142P	H2B 1-19 unmod	H2B 1-19 K5ac	100 ng
B	H2A 1-19 unmod	H2A 1-19 S1P	H2A 1-19 K5ac	H2A 1-19 K9ac	H2A 1-19 K13ac	H2A 110-129 unmod	H2A 110-129 T120P	H2A.X 124-142 unmod	H2A.X 124-142 S139P	H2A.X 124-142 Y142P	H2B 1-19 unmod	H2B 1-19 K5ac	10 ng
C	H2B 1-19 K5me1	H2B 1-19 K12ac	H2B 1-19 S14P	H2B 1-19 K15ac	H2B 107-125 unmod	H2B 107-125 K120ac	H4 1-19 unmod	H4 1-19 S1P	H4 1-19 R3me1	H4 1-19 R3me2a	H4 1-19 R3me2s	H4 1-19 K5ac	100 ng
D	H2B 1-19 K5me1	H2B 1-19 K12ac	H2B 1-19 S14P	H2B 1-19 K15ac	H2B 107-125 unmod	H2B 107-125 K120ac	H4 1-19 unmod	H4 1-19 S1P	H4 1-19 R3me1	H4 1-19 R3me2a	H4 1-19 R3me2s	H4 1-19 K5ac	10 ng
E	H4 1-19 K8ac	H4 1-19 K12ac	H4 11-30 unmod	H4 11-30 K16ac	H4 11-30 R17me1	H4 11-30 R17me2a	H4 11-30 R17me2s	H4 11-30 R19me1	H4 11-30 R19me2a	H4 11-30 R19me2s	H4 11-30 K20ac	H4 11-30 K20me1	100 ng
F	H4 1-19 K8ac	H4 1-19 K12ac	H4 11-30 unmod	H4 11-30 K16ac	H4 11-30 R17me1	H4 11-30 R17me2a	H4 11-30 R17me2s	H4 11-30 R19me1	H4 11-30 R19me2a	H4 11-30 R19me2s	H4 11-30 K20ac	H4 11-30 K20me1	10 ng
G	H4 11-30 K20me2	H4 11-30 K20me3	H4 11-30 R23me1	H4 11-30 R23me2a	H4 11-30 R23me2s	H4 82-100 unmod	H4 82-100 K91ac	100 ng			Rat IgG	Sheep IgG	10 ng
H	H4 11-30 K20me2	H4 11-30 K20me3	H4 11-30 R23me1	H4 11-30 R23me2a	H4 11-30 R23me2s	H4 82-100 unmod	H4 82-100 K91ac	10 ng			Mouse IgG	Rabbit IgG	10 ng

Fig. 2

Dot Blot (Specificity) Analysis:
Representative lot data.
Various modified and non-modified Histone peptides from Absurance™ Histone H3 Antibody Specificity Array (Cat. No. 16-667) (Fig. 1) and from Absurance™ Histone H2A, H2B, H4 Antibody Specificity Array (Cat. No. 16-665) (Fig. 2) were probed with Cat. No. 07-499, Anti-Trimethyl-Histone H3 (Lys27). Proteins were visualized using a Goat Anti-Rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Chromatin Immunoprecipitation (ChIP) Analysis: 1 µg from a representative lot immunoprecipitated Trimethyl-Histone H3 (Lys27) in HeLa and A431 cells.

Fig. 1

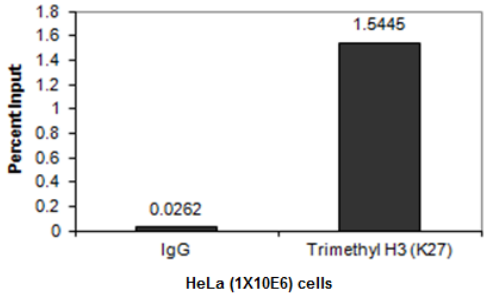
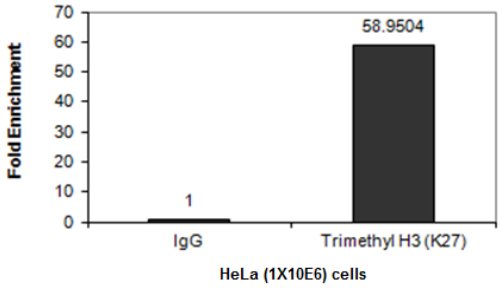


Fig. 2



Chromatin Immunoprecipitation (ChIP) Analysis:
Representative lot data.
Sonicated chromatin was prepared from 1X10E6 equivalents per IP of HeLa cell cells and subjected to chromatin immunoprecipitation using 1 µg of a Goat Anti-Rabbit IgG isotype control or Cat. No. 07-449, Anti-Monomethyl-Histone H3 (Lys26) with the Magna ChIP™ A Kit (Cat. No. 17-610). Successful immunoprecipitation Trimethyl-Histone H3 (Lys27)-associated DNA fragments was verified by qPCR. Data is presented as a percent input of each IP sample relative to input chromatin (Fig. 1) or as fold enrichment of Trimethyl-Histone H3 (Lys27)-associated DNA fragments (Fig. 2)

Immunocytochemistry Analysis: A 1:500 dilution from a representative lot detected Trimethyl-Histone H3 (Lys27) in HeLa and A431 cells.

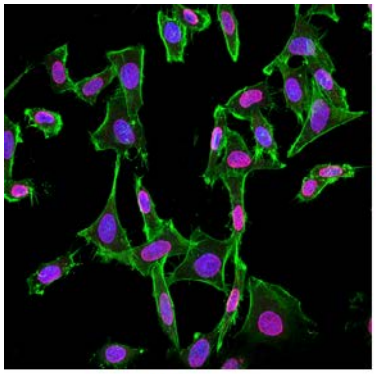


Fig. 1

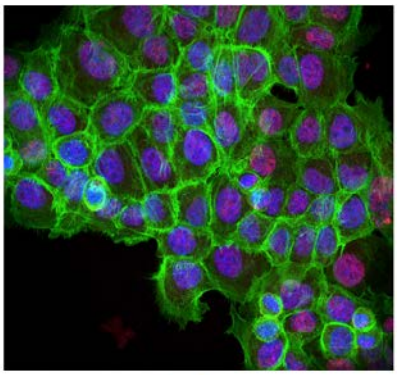
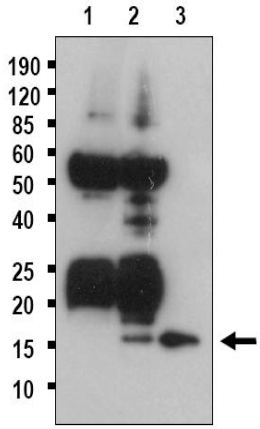


Fig. 2

Immunocytochemistry Analysis
Representative lot data.
Confocal fluorescent analysis of HeLa (Fig. 1) and A431 cells (Fig. 2) using a 1:500 dilution of Cat. No. 07-499, Anti-Trimethyl-Histone H3 (Lys27) and a Goat Anti-Rabbit secondary antibody conjugated to DyLight® 549 Dye (Red). Actin filaments have been labeled with Phalloidin (Green). Nucleus is counterstained with DAPI (Blue, Fig. 1 and 2). This antibody positively stains the nucleus.

DyLight® is a registered trademark of Thermo Fisher Scientific.

Immunoprecipitation Analysis: 4 µg from a representative lot immunoprecipitated Trimethyl-Histone H3 (Lys27) in HeLa acid extract.



Immunoprecipitation Analysis:
Representative lot data.
HeLa acid extracts were probed using 4 µg of rabbit IgG (Lane 1) or with 4 µg of Cat. No. 07-499, Anti-Trimethyl-Histone H3 (Lys27) (Lane 2). Lane 3 represents a 10% input of the IP sample.

Arrow indicates Trimethyl-Histone H3 (Lys27) (~17 kDa).