

Anti-dimethyl-Histone H3 (Lys27)

Polyclonal Antibody

Cat. # 07-452

Lot # 3062774

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

pack size: 200 µg

Store at 2-8°C



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, DB	H	N/A	Dimethylated Lys27	Rb	~17 kDa	Q16695

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 polyclonal in buffer containing 0.1 M Tris-Glycine (pH 7.4) and 150 mM NaCl with 0.05% sodium azide.

Specificity

This antibody recognizes Histone H3 dimethylated at Lys27. Cross-reactivity is observed with dimethyl-Histone H3 (Lys9) due to the presence the conserved ARKS motif. This antibody does not cross react with mono-, tri-, or unmethylated Histone H3.

Species Cross-reactivity

Demonstrated to react with Human.

Immunogen

KLH-conjugated branch peptide corresponding to human Histone H3 dimethylated at Lys27.

Molecular Weight

~17 kDa observed

Method of Purification

Affinity purified

Storage and Handling

Stable for 1 year at 2-8°C from date of receipt.

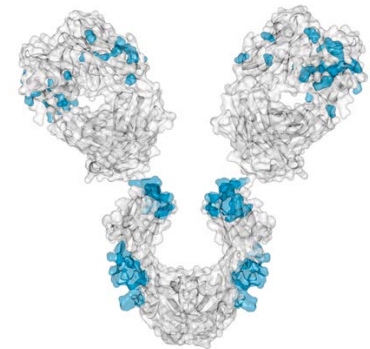
Control

HeLa acid extract

Quality Control Testing

Evaluated by Western Blot in HeLa acid extract.

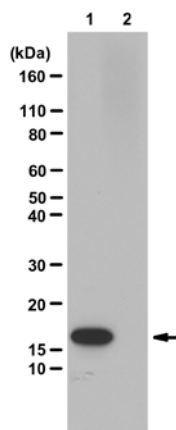
Western Blot Analysis: 0.5 µg/mL of this antibody detected Histone H3 in HeLa acid extract.



References

Background Reference(s):

1. Roudier, F., *et al.* (2011). *EMBO J.* 30(10):1928-1938.
2. Zediak, V.P., *et al.* (2011). *J Immunol.* 186(5): 2705-2709.
3. Kim, Y.M., *et al.* (2011). *Biochem Biophys Res Commun.* 405(2):210-215.
4. Marques, A., *et al.* (2011). *Cytogenet Genome Res.* 134(1):72-82.
5. Ogushi, S., *et al.* (2010). *J Reprod Dev.* 56(5):495-501.



Western Blot Analysis:
Representative lot data:

HeLa acid extract (lane 1) and recombinant Histone H3 (lane 2) were probed with Anti-dimethyl-Histone H3 (Lys27) (0.5 µg/mL). Proteins were visualized using a Donkey Anti-Rabbit IgG secondary antibody conjugated to HRP and a chemiluminescence detection system.

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

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence

IH Immunohistochemistry (Tissue) DB Dot Blot

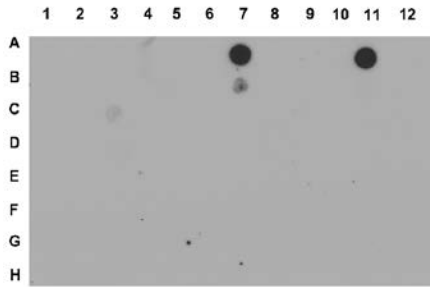
SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates () Predicted Reactivity

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Additional Research Applications:

Dot Bot Analysis: 0.5 µg/mL of this antibody detected dimethyl-Histone H3 (Lys27). Cross-reactivity is observed with dimethyl-Histone H3 (Lys9) due to the presence a conserved ARKS motif. Cross reactivity with mono-, tri-, or unmethylated Histone H3 was not detected.



Dot Blot Analysis:
Representative lot data.

Histone peptides with various modifications (see table below) were transferred to PVDF membrane and probed with Anti-dimethyl-Histone H3 (Lys27). 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)	monomethyl H2A (Lys17)	dimethyl H2A (Lys17)	-	-

PROTOCOL

Western Blot:

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA or milk with 0.05% Tween®-20 surfactant for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of the primary antibody, diluted in freshly prepared 5% BSA or milk for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Visualize with enhanced chemiluminescence (ECL) method of choice.

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RELATED PRODUCTS (specific)

cat #	description
07-369	■ Anti-dimethyl-Histone H3 (Lys36)
07-441	■ Anti-dimethyl-Histone H3 (Lys9)
07-214	■ Anti-dimethyl-Histone H3 (Arg17)
ABE250	■ Anti-dimethyl Histone H3 (Lys4)

RELATED PRODUCTS (non-specific)

cat #	description
WBAVDBASE	■ SNAP i.d.® Protein Detection System
WBAVDABTR	■ SNAP i.d. Antibody Collection Tray
WBAVDR0LL	■ SNAP i.d. Blot Roller
WBAVDBH03	■ SNAP i.d. Triple Well Blot Holder
WBAVDBH01	■ SNAP i.d. Single Well Blot Holder
WBAVDBH02	■ SNAP i.d. Double Well Blot Holder
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
WBLUC0500	■ Luminata Classico Western HRP substrate, 500 mL
WBLUR0500	■ Luminata Crescendo Western HRP substrate, 500 mL

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