Datasheet for ABIN2668415

anti-Histone 3 antibody (H3K9ac)

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Overview

Quantity:	100 µg
Target:	Histone 3 (H3)
Binding Specificity:	H3K9ac
Reactivity:	Human, Mouse
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This Histone 3 antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF), Chromatin Immunoprecipitation (ChIP), Immunocytochemistry (ICC), Dot Blot (DB), ChIP DNA-Sequencing (ChIP-seq), Cleavage Under Targets and Release Using Nuclease (CUT&RUN), Cleavage Under Targets and Tagmentation (CUT&Tag)

Product Details

Immunogen:	This Histone H3 acetylLys9 antibody was raised against a peptide including acetyllysine 9 of histone H3.
Isotype:	IgG
Purification:	Protein A Chromatography

Target Details

Target:	Histone 3 (H3)
Alternative Name:	Histone H3 (H3 Products)

Target Details

Molecular Weight:	17 kDa
Gene ID:	3020

Application Details

Application Notes:	Recommended starting concentrations are ChIP: 10 µg per ChIP ChIP-Seq: 3 µg each ICC/IF: 2 µg/mL dilution WB: 0.5 - 2 µg/mL dilution CUT&RUN: 1:100 CUT&Tag: 1 µg/50 µL reaction CUT&RUN: 2 µL/200 µL reaction Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

Concentration:	1 µg/µL
Buffer:	Purified IgG in PBS (pH 7.5) with 30 % glycerol and 0.035 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Avoid repeated freeze/thaw cycles and keep on ice when not in storage.
Storage:	-20 °C
Storage Comment:	Antibodies in solution can be stored at -20 °C for 2 years.
Expiry Date:	6 months

Publications

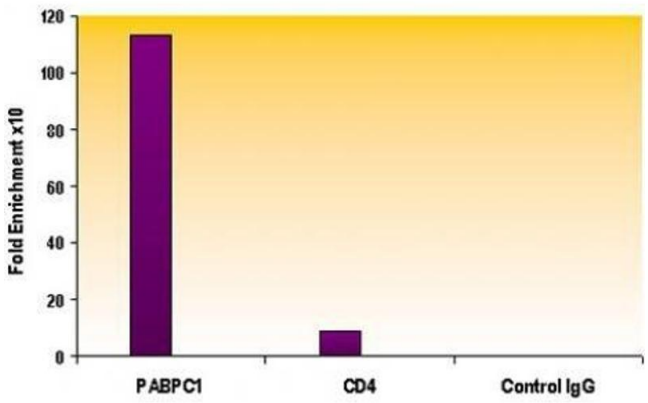
Product cited in:	Zhang, Li, Rezaeian, Xu, Chou, Jin, Han, Pan, Wang, Long, Zhang, Huang, Tsai, Tsai, Logothetis, Lin: "H3 ubiquitination by NEDD4 regulates H3 acetylation and tumorigenesis." in: **Nature communications**, Vol. 8, pp. 14799, (2017) (PubMed).

Publications

Holmberg Olausson, Nistér, Lindström: "Loss of nucleolar histone chaperone NPM1 triggers rearrangement of heterochromatin and synergizes with a deficiency in DNA methyltransferase DNMT3A to drive ribosomal DNA transcription." in: **The Journal of biological chemistry**, Vol. 289, Issue 50, pp. 34601-19, (2014) (PubMed).

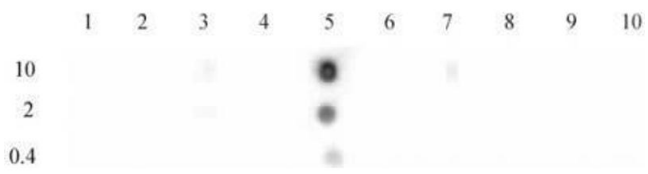
Begum, Stevens, Smith, Connor, Challis, Bloomfield, White: "Epigenetic changes in fetal hypothalamic energy regulating pathways are associated with maternal undernutrition and twinning." in: **FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, Vol. 26, Issue 4, pp. 1694-703, (2012) (PubMed).

Images



Chromatin Immunoprecipitation

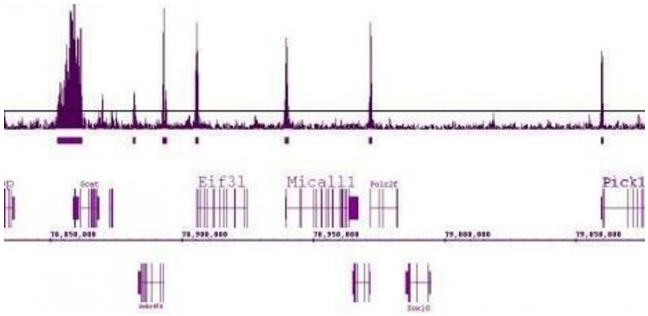
Image 1. ChIP of Histone H3 acetyl Lys9 pAb. Chromatin IP performed using the ChIP-IT® Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 106 cell equivalents per ChIP) using 3 µg of Histone H3 acetyl Lys9 antibody or the equivalent amount of rabbit IgG as a negative control. Real time, quantitative PCR (RT-qPCR) was performed on DNA purified from each of the ChIP reactions using a primer pair specific for the indicated gene. Data are presented as Fold Enrichment of the ChIP antibody signal versus the negative control IgG using the ddCT method.



Dot Blot

Image 2. Histone H3 acetyl Lys9 antibody tested by dot blot analysis. Dot blot analysis was used to confirm the specificity of Histone H3 acetyl Lys9 antibody for acetyl Lys9 histone H3. Acetylated peptides corresponding to the immunogen and related peptides were spotted onto PVDF and probed with Histone H3 acetyl Lys9 antibody at a dilution of 1 µg/ml. The amount of peptide (picomoles) spotted is indicated next to each row. Lane 1: histone H3 acetyl-Lys4 peptide. Lane 2: unmodified Lys4 peptide. Lane

3: acetyl-Lys18 peptide. Lane 4: unmodified Lys9/14/18 peptide. Lane 5: acetyl-Lys9 peptide. Lane 6: acetyl- Lys14 peptide. Lane 7: acetyl-Lys18 peptide. Lane 8: acetyl-Lys23 peptide. Lane 9: acetyl-Lys27 peptide. Lane 10: unmodified Lys27 peptide.



ChIP DNA-Sequencing

Image 3. Histone H3 Acetyl Lys9 antibody (pAb) tested by ChIP-Seq. ChIP was performed using the ChIP-IT® High Sensitivity Kit (Cat. No. 53040) with 30 ug of chromatin from mouse liver. ChIP DNA was sequenced on the Illumina GA II and 25 million sequence tags were mapped to identify H3K9Ac binding across the genome. The image shows a 1.5 million base pair region on chromosome 15. H3K9Ac shows promoter localization at many genes and broader binding near the Gcat gene.

Please check the product details page for more images. Overall 5 images are available for ABIN2668415.

Successfully validated (Cleavage Under Targets and Release Using Nuclease (CUT&RUN))

by Anna Nordin and Claudio Cantù; Cantù Lab, Gene Regulation during Development and Disease, Linköping University

Report Number: 104509

Date: Aug 14 2023

Target:	H3K9ac
Lot Number:	165220066
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Polyclonal rabbit anti-H3K4me (antibodies-online, ABIN3023251)
Negative Control:	Polyclonal guinea pig anti-rabbit IgG (antibodies-online, ABIN101961)
Notes:	Passed. ABIN2667854 allows for specific targeting of H3K9ac in human cells using CUT&RUN.
Primary Antibody:	ABIN2667854

Protocol:

- Cell harvest
  - Harvest 50,000 human fibroblast cells per antibody.
  - Centrifuge cell solution 3 min at 600 x g at RT.
  - Remove the liquid carefully.
  - Gently resuspend cells in 1 mL of Nuclear Extraction Buffer (20 mM HEPES-KOH pH 8.2, 20% Glycerol, 0,05% IGEPAL, 0.5 mM Spermidine, 10 mM KCl, Roche Complete Protease Inhibitor EDTA-free).
  - Move the solution to a 2 mL centrifuge tube.
  - Pellet the nuclei 800 x g for 5 min.
  - Repeat the NE Buffer wash twice for a total of three washes.
  - Resuspend the nuclei in 20 µL NE Buffer per sample.
- Concanavalin A beads preparation
  - Prepare one 2 mL microcentrifuge tube.
  - Gently resuspend the magnetic Concanavalin A Beads (antibodies-online, ABIN6923139).
  - Pipette 10 µL Con A Beads slurry for each sample into the 1.5 mL microcentrifuge tube.
  - Place the tube on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tube from the magnetic stand.
  - Pipette 1 mL Binding Buffer (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$) into each tube and resuspend ConA beads by gentle pipetting.
  - Spin down the liquid from the lid with a quick pulse in a table-top centrifuge.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tube from the magnetic stand.
  - Repeat twice for a total of three washes.
  - Gently resuspend the ConA Beads in a volume of Binding Buffer corresponding to the

original volume of bead slurry, i.e. 10 µL per sample.

- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 10 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly incubates 10 min at 4 °C.
  - Put the 2 mL tube on the magnet stand and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 1 mL of EDTA wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free, 2mM EDTA).
  - Incubate 5 min at RT.
  - Place the tube on the magnet stand and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 200µl of Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) for each sample.
- Cell permeabilization and primary antibody binding
  - Divide nuclei suspension into separate PCR tubes, one for each antibody (200 µL per sample).
  - Add 2 µL antibody (anti-H3K9ac antibody ABIN2667854, anti-H3K4me positive control antibody ABIN3023251, and guinea pig anti-rabbit IgG negative control antibody ABIN101961) to the respective tube, corresponding to a 1:100 dilution.
  - Incubate ON at 4 °C.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Wash with 200 µL of Wash Buffer using a multichannel pipette to accelerate the process.
  - Repeat the wash five times for a total of six washes.
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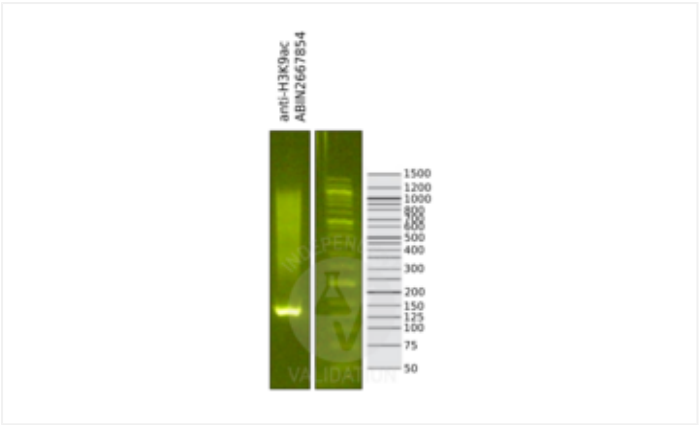
- pAG-MNase Binding
  - Prepare a 1.5 mL microcentrifuge tube containing 200 µL of pAG mix for each sample (200 µl of wash buffer + 120 ng pAG-MNase per sample).
  - Place the PCR tubes with the sample on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove tubes from the magnetic stand.
  - Resuspend the beads in 200 µL of pAG-MNase premix.
  - Incubate for 30 min at 4 °C.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Wash with 200 µL of Wash Buffer using a multichannel pipette to accelerate the process.
  - Repeat the wash for a total of five washes.
  - Resuspend in 200 µL of Wash Buffer.
- MNase digestion and release of pAG-MNase-antibody-chromatin complexes
  - Place PCR tubes on ice and allow to chill.
  - Prepare a 1.5 mL microcentrifuge tube with 51 µl of 2 mM $CaCl_2$ mix per sample (50 µl Wash Buffer + 1 µL 100 mM $CaCl_2$) and let it chill on ice.
  - Always in ice, place the samples on the magnetic rack and when the liquid is clear remove the supernatant.

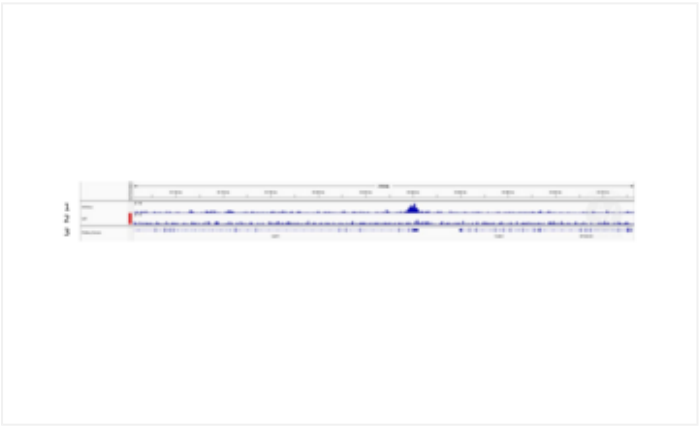
- Resuspend the samples in 50 µl of the 2 mM $CaCl_2$ mix and incubate in ice for exactly 30 min.
- Place the sample on the magnet stand and when the liquid is clear move the supernatant in fresh collection tubes with 3µl of EDTA/EGTA 0.25M (Digestion buffer).
- Resuspend the sample in 47 µl of 1x Urea STOP Buffer (8.5 M Urea, 100 mM NaCl, 2 mM EGTA, 2 mM EDTA, 0,5% IGEPAL).
- Incubate the samples 1 h at 4 °C.
- Transfer the supernatant containing the pAG-MNase-bound digested chromatin fragments to the previously collected digestion buffer.

- DNA Clean up
  - Take the Mag-Bind® TotalPure NGS beads (Omega Bio-Tek, M1378-01) from the storage and wait until they are at RT.
  - Add 2x volume of beads to each sample (e.g. 100 µL of beads for 50 µL of sample).
  - Incubate the beads and the sample for 15 min at RT.
  - During incubation prepare fresh EtOH 80%.
  - Place the PCR tubes on a magnet stand and when the liquid is clear remove the supernatant.
  - Add 200 µl of fresh 80% EtOH to the sample without disturbing the beads (Important!!! Do NOT resuspend the beads or remove the tubes from the magnet stand or the sample will be lost).
  - Incubate 30 sec at RT.
  - Remove the EtOH from the sample.
  - Repeat the wash with 80% EtOH.
  - Resuspend the beads in 25 µL of 10 mM Tris.
  - Incubate the sample for 2 min at RT.
  - Repeat the 2x beads clean up as described before (this time with 50 µL of beads for each sample).
  - Resuspend the beads + DNA in 20 µL of 10 mM Tris.
- Library preparation and sequencing
  - Prepare libraries using KAPA HyperPrep Kit using KAPA Dual-Indexed adapters according to protocol.
  - Sequence samples on an Illumina NextSeq 500 sequencer, using a NextSeq 500/550 High Output Kit v2.5 (75 Cycles), 36bp PE.
- Bioinformatics
  - Align reads the human genome (hg38) using bowtie78 with settings -X 700 -m1 -v 3. Remove duplicate reads, and sort files using samtools. Filter mapped reads for size, keeping only reads with a fragment size at or below 120 base pairs.
  - Generate bedgraph files using bedtools genomecov.
  - Call peaks using SEACR version 1.3, in relaxed mode, normalizing to the negative control.



Validation image no. 1 for anti-Histone 3 (H3) (H3K9ac) antibody (ABIN2667854)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-H3K9ac antibody ABIN2667854 (left) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher) (right).



Validation image no. 2 for anti-Histone 3 (H3) (H3K9ac) antibody (ABIN2667854)

1. Alignment tracks from CUT&RUN targeting H3K9ac in human fibroblast cells using antibody ABIN2667854 showing the VMP1 locus. 2. Alignment tracks for CUT&RUN with the IgG negative control ABIN101961. 3. RefSeq Genes.