Datasheet for ABIN3023251

anti-Histone 3 antibody (H3K4me)

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Overview

Quantity:	100 µL
Target:	Histone 3 (H3)
Binding Specificity:	H3K4me
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This Histone 3 antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF), Chromatin Immunoprecipitation (ChIP), Immunoprecipitation (IP), ChIP DNA-Sequencing (ChIP-seq), Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

Product Details

Immunogen:	A synthetic methylated peptide corresponding to residues surrounding K4 of human histone H3
Isotype:	IgG
Cross-Reactivity:	Human, Mouse, Rat
Characteristics:	Methylated Antibodies
Purification:	Affinity purification

Target Details

Target:	Histone 3 (H3)

Target Details

Alternative Name:	Histone H3 (H3 Products)
Background:	Histones are basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes. Nucleosomes consist of approximately 146 bp of DNA wrapped around a histone octamer composed of pairs of each of the four core histones (H2A, H2B, H3, and H4). The chromatin fiber is further compacted through the interaction of a linker histone, H1, with the DNA between the nucleosomes to form higher order chromatin structures. This gene is intronless and encodes a replication-dependent histone that is a member of the histone H3 family. Transcripts from this gene lack polyA tails, instead, they contain a palindromic termination element. This gene is located separately from the other H3 genes that are in the histone gene cluster on chromosome 6p22-p21.3.,H3.4,H3/g,H3FT,H3t,HIST3H3,Histone H3,HIST1H3A,Signal Transduction,MAPK-Erk Signaling Pathway,MAPK-P38 Signaling Pathway,Epigenetics & Nuclear Signaling,Epigenetic Modifications,Methylation,Histone H3
Molecular Weight:	15 kDa
Gene ID:	8290
UniProt:	Q16695

Application Details

Application Notes:	WB 1:500 - 1:2000, IF 1:50 - 1:200, IP 1:50 - 1:200, ChIP 1:50 - 1:200, ChIP-seq 1:50 - 1:200, CUT&RUN 1:100
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	PBS with 0.02 % sodium azide,50 % glycerol, pH 7.3.
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 freeze / thaw cycles
Storage:	-20 °C
Storage Comment:	Store at -20°C. Avoid freeze / thaw cycles.

Publications

Product cited in:

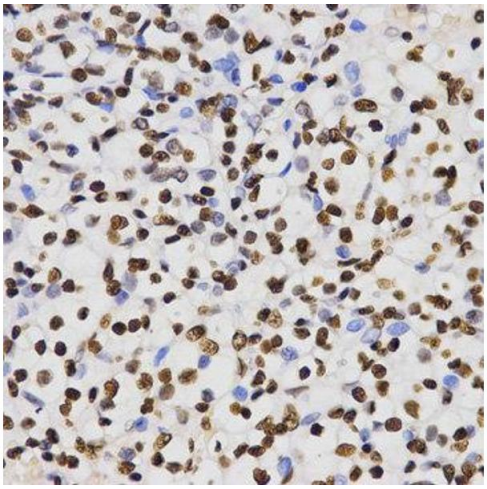
Zambanini, Nordin, Jonasson, Pagella, Cantù: "A new cut&run low volume-urea (LoV-U) protocol optimized for transcriptional co-factors uncovers Wnt/b-catenin tissue-specific genomic targets." in: **Development (Cambridge, England)**, (2022) (PubMed).

Zhang, Zhang, Cheng, Liu, Lin, Wu, Zhang, Wang, Wang, Guo, Zhang, Lei, Zhao, Zhu, Wan: " Functional characterization of rice CW-domain containing zinc finger proteins involved in histone recognition." in: **Plant science : an international journal of experimental plant biology**, Vol. 263, pp. 168-176, (2018) (PubMed).

Chen, Zhang, Li, Feng, Zhang, Yao, Zhang, Wan: "Celastrol attenuates incision-induced inflammation and pain associated with inhibition of the NF-κB signalling pathway via SARM." in: **Life sciences**, Vol. 205, pp. 136-144, (2018) (PubMed).

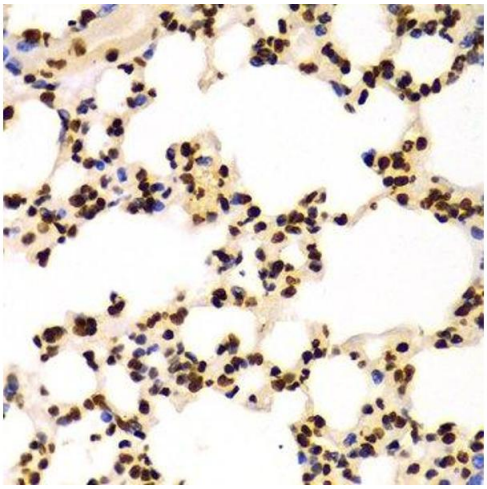
Cao, Liu, Yue, Liu, Pei, Gu, Wang, Jia: "Iron chelation inhibits cancer cell growth and modulates global histone methylation status in colorectal cancer." in: **Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine**, Vol. 31, Issue 5, pp. 797-805, (2018) (PubMed).

Images



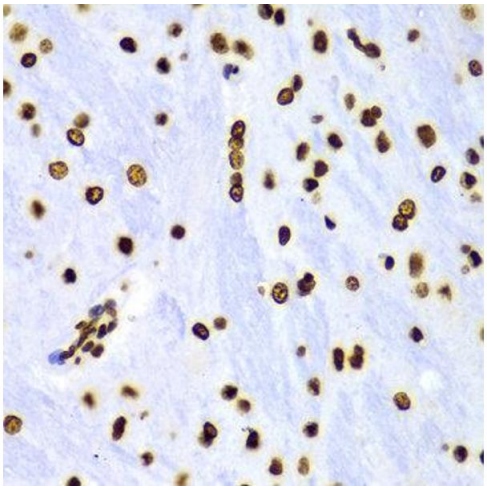
Immunohistochemistry

Image 1. Immunohistochemistry of paraffin-embedded human kidney cancer tissue using H3K4me2 antibody at dilution of 1:200 (x400 lens).



Immunohistochemistry (Paraffin-embedded Sections)

Image 2. Immunohistochemistry of paraffin-embedded Mouse lung using DiMethyl-Histone H3-K4 antibody.



Immunohistochemistry

Image 3. Immunohistochemistry of paraffin-embedded Rat brain using H3K4me2 antibody at dilution of 1:100 (x400 lens).

Please check the product details page for more images. Overall 21 images are available for ABIN3023251.

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

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

Report Number: 104228

Date: Nov 12 2021

Target:	H3K4me
Lot Number:	3560036504
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Recombinant anti-H3K27me3 CUT&RUN Positive Control antibody (antibodies-online, ABIN6923144)
Negative Control:	Guinea Pig anti-rabbit IgG (antibodies-online, ABIN101961)
Notes:	Passed. ABIN3023251 allows for H3K4me targeted digestion using CUT&RUN.
Primary Antibody:	ABIN3023251
Protocol:	• Cell harvest ◦ Harvest 250,000 HEK293T cells per antibody to be used at RT. ◦ Centrifuge cell solution 3 min at 600 x g at RT. ◦ Remove the liquid carefully. ◦ Gently resuspend cells in 1 mL Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) by pipetting and transfer cell solution to a 2 mL microcentrifuge tube. ◦ Centrifuge cell solution 3 min at 600 x g at RT and discard the supernatant. ◦ Repeat twice for a total of three washes. ◦ Resuspend cell pellet in 1 mL Wash Buffer by gently pipetting. • Concanavalin A beads preparation ◦ Prepare one 1.5 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.

- Cell immobilization – binding to Concanavalin A beads
  - Carefully vortex the cell suspension and add 10 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly and rotate for 10 min at RT.
- Cell permeabilization and primary antibody binding
  - Divide cell suspension into separate 2 mL microcentrifuge tubes, one for each antibody (250,000 cells per sample).
  - Place the microcentrifuge tubes on a magnetic stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 150 µL Digitonin Wash buffer (wash buffer with 0.025% (wt/vol) Digitonin) supplemented with 2 mM EDTA.
  - Gently vortex the microcentrifuge tubes until the beads are resuspended.
  - o Add 1.5 µL antibody (anti-H3K4me ABIN3023251, anti-H3K27me3 positive control antibody ABIN6923144, or guinea pig anti-rabbit IgG negative control antibody ABIN101961) to the respective tube, corresponding to a 1:100 dilution.
  - Rotate the microcentrifuge tubes ON at 4 °C.
  - Spin down the liquid and place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 ml pipette tip.
  - Repeat once for a total of two washes.
- pA-MNase Binding
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Vortex the sample at low speed and add 150 µL CUTANA pAG-MNase 0.5X (antibodies-online ABIN6950951, 1:40 dilution of a 20X stock in Digitonin Wash Buffer) per sample, gently resuspending the beads by pipetting.
  - Rotate the microcentrifuge tubes for 1 h at 4 °C.
  - Spin down the liquid and place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 mL pipette tip.
  - Repeat once for a total of two washes.
- MNase digestion and release of pA-MNase-antibody-chromatin complexes
  - 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.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 100 µL

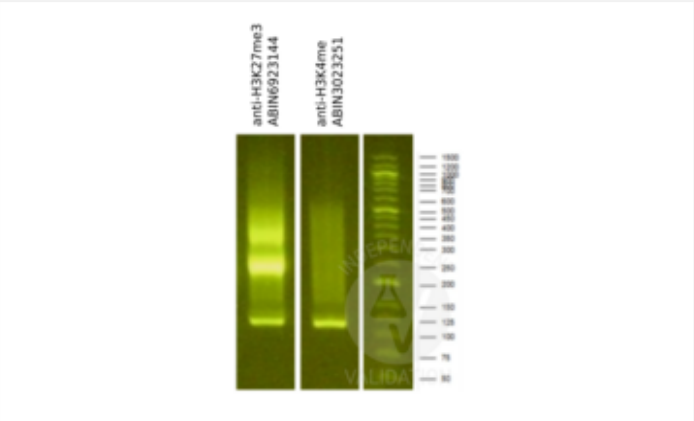
Digitonin Wash buffer per sample along the side of the tube.
- Place tubes in a heat block, kept on ice, and allow to chill.
- Add 2 µL 0.1 M CaCl2 to each sample.
- Incubate tubes at 0 °C for 15 min.
- Add 100 µL 2X STOP buffer (340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 0.05% (wt/vol) Digitonin, 100 µg/mL RNAse A, 50 µg/mL Glycogen).
- Incubate tubes at 37 °C for 30 min.
- Place the tubes on a magnet stand until the fluid is clear.
- Transfer the supernatant containing the pAG-MNase-bound digested chromatin fragments to fresh 1.5 mL microcentrifuge tubes.

- DNA extraction
  - Add 2 µL 10% SDS to a final concentration of 0.1% and 2.5 µL Proteinase K (20 mg/mL) to a final concentration of 0.25 mg/mL to each supernatant.
  - Gently vortex tubes at a low speed of approximately 1,100 rpm.
  - Incubate tubes at 50 °C for 1 h.
  - Add 200 µL PCI to tube.
  - Vortex tubes thoroughly at high speed until the liquid appears milky.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at RT for 5 min.
  - Carefully transfer to upper aqueous phase to a fresh 1.5 mL microcentrifuge tube containing 2 µL glycogen (diluted 1:10 to 2 mg/mL from the 20 mg/mL stock solution).
  - Add 20 µL 3 M NaOAc pH 5.2.
  - Add 400 µL 100% ethanol.
  - Place tubes for at -20 °C ON.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 5min.
  - Remove the liquid carefully with a pipette.
  - Wash pellet with 1ml 70% ethanol.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 1 min.
  - Remove the liquid carefully with a pipette.
  - Air-dry the pellet, then dissolve in 30 µL 1 mM Tris-HCl, 0.1 mM EDTA.
- 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.
- Peak calling
  - Trim reads using bbTools bbduk to remove adapters, artifacts and repeat sequences.
  - Aligned reads were mapped to the GRCh38 (hg38) human genome using bowtie2 with options --local --very-sensitive- local --no-unal --no-mixed --no-discordant - X 400.
  - Convert SAM files to BAM files and remove duplicates using SAMtools was used to convert SAM files to BAM files. Produce Bedgraph files with BEDtools genomecov.
  - Call peaks using SEACR against the IgG negative control with the options norm and relaxed.

Experimental Notes:	The CUT&RUN alignment track was compared to a reference alignment track of ChIP-seq for

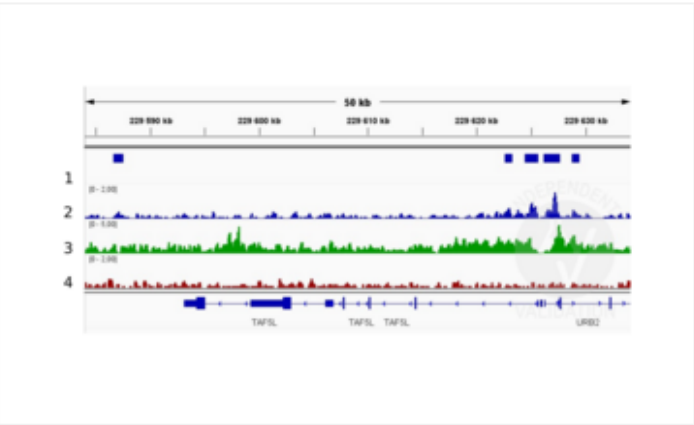
H3K4me in HEK293 cells obtained from ENCODE (PMID 26527727), experiment ENCSR000FCG, track ENCFF274LAP.

Images for Validation report #104228



Validation image no. 1 for anti-Histone 3 (H3) (H3K4me) antibody (ABIN3023251)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using an anti-H3K27me3 CUT&RUN Positive Control antibody (ABIN6923144) and anti-H3K4me (ABIN3023251) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher).



Validation image no. 2 for anti-Histone 3 (H3) (H3K4me) antibody (ABIN3023251)

Alignment tracks from CUT&RUN targeting H3K4me in HEK293T cells. 1. Peaks called by SEACR from CUT&RUN data using anti-H3K4me antibody ABIN3023251. 2. Alignment track for CUT&RUN reads obtained using anti-H3K4me antibody ABIN3023251 in HEK293T cells. CUT&RUN reads are normalized to sequencing depth per million reads. 3. Alignment track of ChIP-seq for H3K4me in HEK293 cells obtained from ENCODE, experiment ENCSR000FCG, track ENCFF274LAP. Coverage is shown as fold change over control. 4. Alignment track for CUT&RUN negative control normalized to sequencing depth per million reads.