Datasheet for ABIN2668403

anti-Histone 3 antibody (H3K36me3)

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Overview

Quantity:	100 µg
Target:	Histone 3 (H3)
Binding Specificity:	H3K36me3
Reactivity:	Human, Mouse
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Histone 3 antibody is un-conjugated
Application:	Western Blotting (WB), Chromatin Immunoprecipitation (ChIP), 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 trimethylLys36 antibody was raised against a peptide containing trimethylLys36 of human Histone H3.
Clone:	MABI 0333
Isotype:	IgG1
Purification:	Protein G 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: 5 - 10 µg per ChIP ChIP-Seq: 5 - 10 µg each WB: 0.5 - 2 µg/mL dilution DB: 0.5 - 2 µg/mL dilution CUT&RUN: 2 µL/200 µL reaction Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

Concentration:	0.44 µg/µL
Buffer:	PBS pH 7.5 containing 30 % glycerol, 0.3 M NaCl, 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

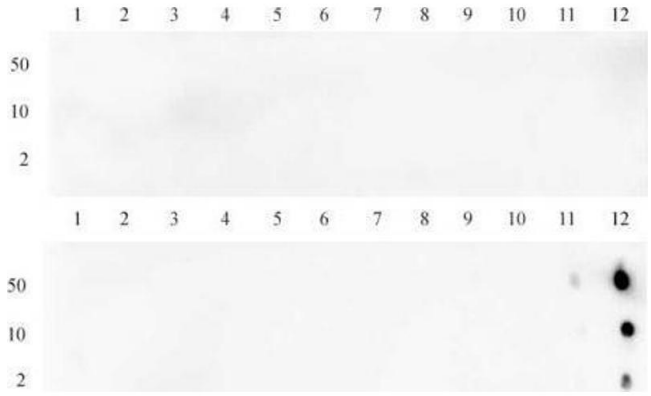
Product cited in:	Brahma, Henikoff: "RSC-Associated Subnucleosomes Define MNase-Sensitive Promoters in Yeast." in: **Molecular cell**, Vol. 73, Issue 2, pp. 238-249.e3, (2019) (PubMed). Powers, Parvanov, Baker, Walker, Petkov, Paigen: "The Meiotic Recombination Activator PRDM9 Trimethylates Both H3K36 and H3K4 at Recombination Hotspots In Vivo." in: **PLoS genetics**, Vol. 12, Issue 6, pp. e1006146, (2016) (PubMed).

Publications

Xu, Gan, Zhou, Wee, Zhang, Ito: "Arabidopsis MRG domain proteins bridge two histone modifications to elevate expression of flowering genes." in: **Nucleic acids research**, Vol. 42, Issue 17, pp. 10960-74, (2014) (PubMed).

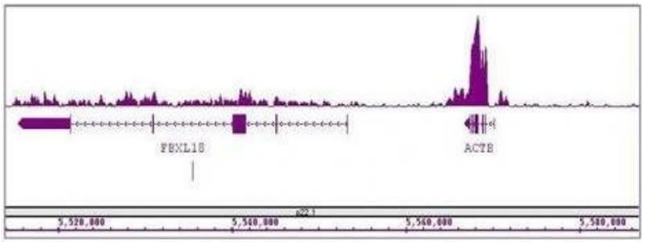
Rechtsteiner, Ercan, Takasaki, Phippen, Egelhofer, Wang, Kimura, Lieb, Strome: "The histone H3K36 methyltransferase MES-4 acts epigenetically to transmit the memory of germline gene expression to progeny." in: **PLoS genetics**, Vol. 6, Issue 9, pp. e1001091, (2010) (PubMed).

Images



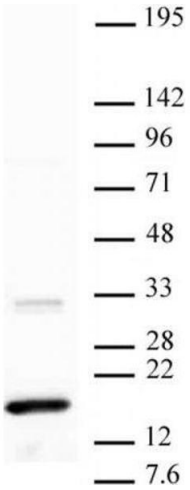
Dot Blot

Image 1. Dot blot of Histone H3 trimethyl Lys36 antibody. Dot blot analysis was used to confirm the specificity of Histone H3 trimethyl Lys36 antibody for trimethyl Lys36 of histone H3. Recombinant methylated peptides corresponding to the immunogen and related sequences were spotted onto PVDF and probed with Histone H3 trimethyl Lys36 at 2 µg/ml. The amount of protein (picomoles) spotted is indicated next to each row. Top panel - Lane 1: unmodified H3 Lys4. Lane 2: monomethyl Lys4. Lane 3: dimethyl Lys4. Lane 4: trimethyl Lys4. Lane 5: unmodified Lys9. Lane 6: monomethyl Lys9. Lane 7: dimethyl Lys9. Lane 8: trimethyl Lys9. Lane 9: unmodified Lys79. Lane 10: monomethyl Lys79. Lane 11: dimethyl Lys79. Lane 12: trimethyl Lys79. Bottom panel - Lane 1: unmodified H3 Lys23. Lane 2: monomethyl Lys23. Lane 3: dimethyl Lys23. Lane 4: trimethyl Lys23. Lane 5: unmodified Lys27. Lane 6: monomethyl Lys27. Lane 7: dimethyl Lys27. Lane 8: trimethyl Lys27. Lane 9: unmodified Lys36. Lane 10: monomethyl Lys36. Lane 11: dimethyl Lys36. Lane 12: trimethyl Lys36.



ChIP DNA-Sequencing

Image 2. Histone H3K36me3 antibody (mAb) tested by ChIP-Seq. ChIP was performed using the ChIP-IT® High Sensitivity Kit (Cat. No. 53040) with 15 ug of chromatin from a human medulloblastoma cell line and 4 µg of antibody. ChIP DNA was sequenced on the Illumina HiSeq and 15 million sequence tags were mapped to identify Histone H3K36me3 binding sites. The image shows binding across a region of chromosome 7. You can view the complete data set in the UCSC Genome Browser, starting at this specific location, here.



Western Blotting

Image 3. Western blot of Histone H3 trimethyl Lys36 antibody. HeLa nuclear extract (20 µg per lane) probed with Histone H3 trimethyl Lys36 antibody (2 µg/ml dilution).

Please check the product details page for more images. Overall 5 images are available for ABIN2668403.

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: 104510

Date: Aug 14 2023

Target:	H3K36me3
Lot Number:	20822015
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. ABIN2668403 allows for specific targeting of H3K36me3 in human cells using CUT&RUN.
Primary Antibody:	ABIN2668403

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-H3K36me3 antibody ABIN2668403, 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.
- 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) (H3K36me3) antibody (ABIN2668403)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-H3K36me3 antibody ABIN2668403 (left) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher) (right).

Validation image no. 2 for anti-Histone 3 (H3) (H3K36me3) antibody (ABIN2668403)

1. Alignment tracks from CUT&RUN targeting H3K36me3 in human fibroblast cells using antibody ABIN2668403 showing the USP32 locus. 2. Alignment tracks for CUT&RUN with the IgG negative control ABIN101961. 3. RefSeq Genes.