Datasheet for ABIN6971977

anti-Histone 3 antibody (3meLys4)

1 Validation

4 Images

Go to Product page

Overview

Quantity:	100 µg
Target:	Histone 3 (H3)
Binding Specificity:	3meLys4
Reactivity:	Human, Saccharomyces cerevisiae
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)

Product Details

Immunogen:	This Histone H3 trimethyl Lys4 antibody was raised against a peptide including trimethyl-lysine 4 of histone H3.
Isotype:	IgG
Characteristics:	Histone H3 is one of the core components of the nucleosome. The nucleosome is the smallest subunit of chromatin and consists of 147 base pairs of DNA wrapped around an octamer of core histone proteins (two each of Histone H2A, Histone H2B, Histone H3 and Histone H4). Histone H1 is a linker histone, present at the interface between the nucleosome core and DNA entry/exit points. Histone H1 is responsible for establishing higher-order chromatin structure. Chromatin is subject to a variety of chemical modifications, including post-translational modifications of the histone proteins and the methylation of cytosine residues in the DNA.

Product Details

	Reported histone modifications include acetylation, methylation, phosphorylation, ubiquitylation, glycosylation, ADP-ribosylation, carbonylation and SUMOylation, these modifications play a major role in regulating gene expression. The methylation of histones can occur on two different residues: arginine or lysine. Histone methylation can be associated with transcriptional activation or repression, depending on the methylated residue. Lysine 4 of histone H3 can be mono-, di- or trimethylated by different histone methyltransferases (HMTs) such as SET1 or ASH1. Methylation of Lys4 is often associated with transcriptional activation. The demethylase LSD1 is able to demethylate histone H3 Lys4. Histone H3K4me3 antibody (pAb) was raised in a Rabbit host. It has been validated for use in Chromatin Immunoprecipitation, ChIP-Seq, Dot blot, Immunocytochemistry, Immunofluorescence and Western blot, it has been shown to react with Budding Yeast and Human samples, but it is predicted that it will react with a wide range of sample types.
Purification:	Protein A Chromatography

Target Details

Target:	Histone 3 (H3)
Alternative Name:	Histone H3 (H3 Products)
Molecular Weight:	17 kDa
NCBI Accession:	NP_003522

Application Details

Application Notes:	Recommended starting concentrations are ChIP: 3 µg per ChIP ChIP-Seq: 4 µg each ICC/IF: 1 - 2 µg/mL dilution WB: 2 - 4 µg/mL dilution CUT&RUN: 1:100 Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

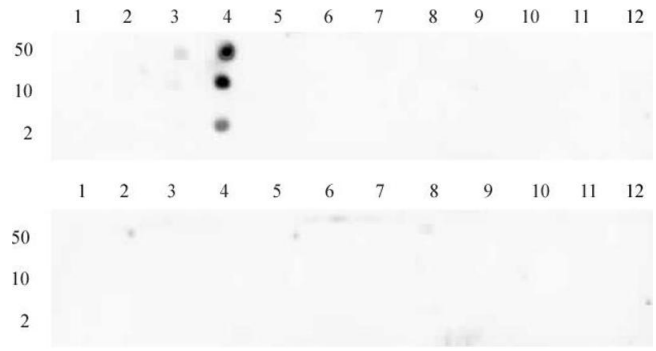
Handling

Buffer:	Purified IgG in PBS (pH 7.5) with 30 % glycerol and 0.035 % sodium azide.

Handling

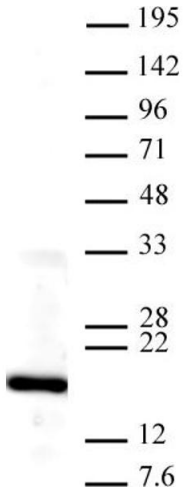
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage.

Images



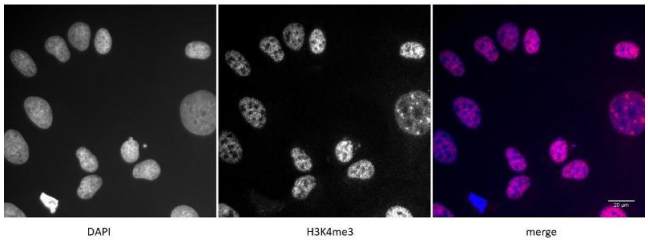
Dot Blot

Image 1. Histone H3 trimethyl Lys4 antibody tested by dot blot analysis. Dot blot analysis was used to confirm the specificity of Histone H3 trimethyl Lys4 antibody for trimethyl-Lys4 of histone H3. Peptides corresponding to regions around major sites of histone H3 methylation were spotted onto PVDF and probed with Histone H3 trimethyl Lys4 antibody at a dilution of 1 µg/mL . The amount of peptide (in picomoles) spotted is indicated next to each row. Top panel: Lane 1: unmodified Lys4. Lane 2: monomethyl Lys4. Lane 3: dimethyl Lys4. Lane 4: trimethyl Lys4. Lane 5: unmodified Lys9, 14, 18. Lane 6: monomethyl Lys9. Lane 7: dimethyl Lys9. Lane 8: trimethyl Lys9. Lane 9: dimethyl Lys14. Lane 10: monomethyl Lys18. Lane 11: dimethyl Lys18. Lane 12: trimethyl Lys18. Bottom panel: Lane 1: Unmodified 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.



Western Blotting

Image 2. Histone H3K4me3 antibody tested by Western blot. The analysis was performed using 10 µg HeLa nuclear cell extract and Histone H3K4me3 antibody at 2 µg/mL. *For optimal results, we recommend a High Salt & Sonication Protocol when preparing nuclear extracts. Visit activemotif.com to download the protocol.



Immunofluorescence

Image 3. Detection of H3K4me3 by immunofluorescence U2OS cells were stained with H3K4me3 antibody at a dilution of 1:500. Left panel: DAPI. Middle panel: H3K4me3 antibody staining Right panel: merge.

Please check the product details page for more images. Overall 4 images are available for ABIN6971977.

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

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

Report Number: 104467

Date: Apr 26 2023

Target:	H3K4me3
Lot Number:	30720008
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. The anti-H3K4me3 antibody ABIN6971977 allows for H3K4me3 targeted digestion using CUT&RUN in mouse fore limb (11.5) cells.
Primary Antibody:	ABIN6971977

Protocol:

- Cell harvest and nuclear extraction
  - Dissect 3 Fore limbs (11.5 DAC) from RjOrl:SWISS embryos for each sample.
  - Dissociate the tissue into single cells in TrypLE for 15 min at 37 °C.
  - Centrifuge cell solution 5 min at 800 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 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, ABIN6952467).
  - Pipette 20 µL Con A Beads slurry for each sample into the 2 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 the 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 the wash 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. 20 µL per sample.

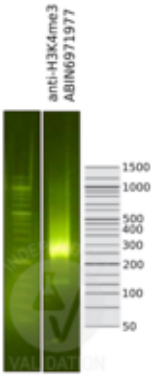
- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 20 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly incubates 10 min at 4 °C.
  - Put the 1.5 mL tube on the magnet rack and when the liquid 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, 2 mM EDTA).
  - Incubate for 5 min at RT.
  - Place the tube on the magnet stand and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 200 µL of Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) per sample.
- Primary antibody binding
  - Divide nuclei suspension into separate 200 µL PCR tubes, one for each antibody (150,000 cells per sample).
  - Add 2 µL antibody (anti H3K4me3 antibody ABIN6971977, anti-H3K4me positive control antibody ABIN3023251, 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 (to accelerate the process use a multichannel pipette).
  - Repeat the wash for a total of five washes.
- pAG-MNase Binding
  - Prepare a 1.5 mL microcentrifuge tube containing 200 µL of pAG mix pear 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 CaCl2 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 3 µL of EDTA/EGTA 0.25 M (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 for 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 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.
    - 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 and 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), 36 bp PE.
- Peak calling
    - Trim reads using using bbTools bbduk (BBMap - Bushnell B. - sourceforge.net/projects/bbmap/) to remove adapters, artifacts and repeat sequences.
    - Map aligned reads to the mm10 mouse genome using bowtie with options -m 1 -v 0 -l 0 -X 500.
    - Use SAMtools to convert SAM files to BAM files and remove duplicates.
    - Use BEDtools genomecov to produce Bedgraph files.
    - Call peaks using SEACR with a 0.001 threshold and the option norm stringent.

Experimental Notes:	The protocol is published in Zambanini, G. et al. A New CUT&RUN Low Volume-Urea (LoV-U)

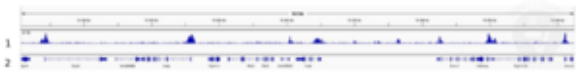
protocol uncovers Wnt/β-catenin tissue-specific genomic targets. Development (2022). PMID 36355069

Images for Validation report #104467



Validation image no. 1 for anti-Histone 3 (H3) (3meLys4) antibody (ABIN6971977)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-H3K4me3 antibody ABIN6971977 and anti- after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher).



Validation image no. 2 for anti-Histone 3 (H3) (3meLys4) antibody (ABIN6971977)

1. Alignment tracks from CUT&RUN targeting H3K4me3 in mouse fore limb (11.5) cells using anti-H3K4me3 antibody ABIN6971977. 2. RefSeq Genes.