Datasheet for ABIN2667903

anti-Histone 3 antibody (H3K27ac)

1 Validation

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Overview

Quantity:	10 µg
Target:	Histone 3 (H3)
Binding Specificity:	H3K27ac
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), Cleavage Under Targets and Tagmentation (CUT&Tag)

Product Details

Isotype:	IgG
Purification:	Protein A Chromatography

Target Details

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

Application Details

Application Notes:	This rabbit anti-H3K27ac antibody is suitable for use in CUT&RUN, CUT&Tag, ChIP-seq, Chromatin Immunoprecipitation, , Dot Blot, Immunocytochemistry, Immunofluorescence, and Western Blot. Specific conditions for each assay should be optimized by the end user. General dilution recommendations for different applications are as follows: CUT&RUN: 1:100 CUT&Tag: 1:100 ChIP: 10 µg/ChIP ChIP-seq: 5 µg/ChIP ICC: 1-5 µg/ml IF: 1-5 µg/ml WB: 0.1-1 µg/ml
Restrictions:	For Research Use only

Handling

Concentration:	1 µg/µL
Buffer:	Purified IgG in PBS 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. This product is guaranteed for 6 months from date of receipt.
Storage:	-20 °C
Storage Comment:	Antibodies in solution can be stored at -20°C for 2 years.
Expiry Date:	6 months

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

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Report Number: 104485

Date: Jun 09 2022

Target:	H3K27ac
Lot Number:	6921014
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. ABIN2667903 allows for H3K27Ac targeted digestion using CUT&RUN in mouse fore limb (11.5) cells.
Primary Antibody:	ABIN2667903

Protocol:

- Cell harvest and nuclear extraction
  - Dissect 3 Fore limbs (11.5 DAC) from mouse strain RjOrl:SWISS 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.

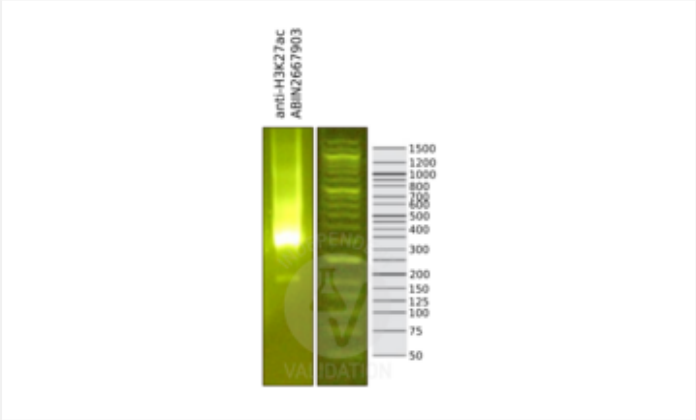
- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 20 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly incubates 10 min at 4 °C.
  - Put the 2 mL tube on the magnet stand and when the liquid 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 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-H3K27ac antibody ABIN2667903, 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 at 4 °C ON.
  - 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 100 µL of pAG mix per sample (100 µL of wash buffer + 58.5 µg 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 100 µL of pAG-MNase premix.
  - Incubate 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 five times for a total of six washes.
  - Resuspend in 100 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 102 µl of 2 mM $CaCl_2$ mix per sample (100 µl

Wash Buffer + 2 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 100 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 remove the supernatant.
    - Resuspend the sample in 50 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 1h at 4°C.
    - Transfer the supernatant containing the pAG-MNase-bound digested chromatin fragments to fresh 200 µl PCR tubes.
- 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-HCl pH 8.2.
    - 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-HCl pH 8.2.
- 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 hg38 human 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.

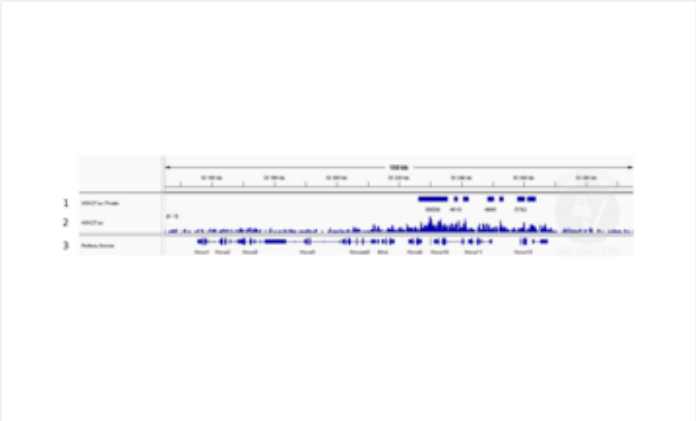
Experimental Notes:	The protocol is published in PMID 36355069 .

Images for Validation report #104485



Validation image no. 1 for anti-Histone 3 (H3) (H3K27ac) antibody (ABIN2667903)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-H3K27ac antibody ABIN2667903 after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher).



Validation image no. 2 for anti-Histone 3 (H3) (H3K27ac) antibody (ABIN2667903)

1. Peaks called by SEACR from CUT&RUN data using anti-H3K27ac antibody ABIN2667903. 2. Alignment tracks from CUT&RUN targeting H3K27ac in mouse fore limb (11.5) cells using anti-H3K27ac antibody ABIN2667903 showing the Hoxa locus 3. RefSeq Genes.