### Overview

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

#### Product Details

<table>
<thead>
<tr>
<th><strong>Immunogen:</strong></th>
<th>A synthetic methylated peptide corresponding to residues surrounding K4 of human histone H3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isotype:</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Cross-Reactivity:</strong></td>
<td>Human, Mouse, Rat</td>
</tr>
<tr>
<td><strong>Characteristics:</strong></td>
<td>Methylated Antibodies</td>
</tr>
<tr>
<td><strong>Purification:</strong></td>
<td>Affinity purification</td>
</tr>
</tbody>
</table>

#### 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.

**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


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).
Images

Please check the product details page for more images. Overall 21 images are available for ABIN3023251.
# Validation report #104228 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

**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

<table>
<thead>
<tr>
<th>Target:</th>
<th>H3K4me</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot Number:</td>
<td>3560036504</td>
</tr>
<tr>
<td>Method validated:</td>
<td>Cleavage Under Targets and Release Using Nuclease (CUT&amp;RUN)</td>
</tr>
<tr>
<td>Positive Control:</td>
<td>Recombinant anti-H3K27me3 CUT&amp;RUN Positive Control antibody (antibodies-online, ABIN6923144)</td>
</tr>
<tr>
<td>Negative Control:</td>
<td>Guinea Pig anti-rabbit IgG (antibodies-online, ABIN101961)</td>
</tr>
<tr>
<td>Notes:</td>
<td>Passed. ABIN3023251 allows for H3K4me targeted digestion using CUT&amp;RUN.</td>
</tr>
<tr>
<td>Primary Antibody:</td>
<td>ABIN3023251</td>
</tr>
</tbody>
</table>

## 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 CaCl₂, 1 mM MnCl₂) 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.

International: +49 (0)241 95 163 153 | USA & Canada: +1 877 302 8632 | support@antibodies-online.com  
Page 5/8 | Product datasheet for ABIN3023251 | 09/11/2023 | Copyright antibodies-online. All rights reserved.
Validation report #104228 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

- 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.
- 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
Validation report #104228 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

1. 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.

2. DNA extraction
   ○ Add 2 μL 10% SDS to a final concentration of 0.1% and 2.5 μL Proteinase K (20 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 5 min.
   ○ Remove the liquid carefully with a pipette.
   ○ Wash pellet with 1 ml 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.

3. 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.

4. 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
Validation report #104228 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

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.