**anti-Histone 3 antibody (H3K36me3)**

### Overview

<table>
<thead>
<tr>
<th>Quantity</th>
<th>100 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Histone 3 (H3)</td>
</tr>
<tr>
<td>Binding Specificity</td>
<td>H3K36me3</td>
</tr>
<tr>
<td>Reactivity</td>
<td>Human, Mouse</td>
</tr>
<tr>
<td>Host</td>
<td>Mouse</td>
</tr>
<tr>
<td>Clonality</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Application</td>
<td>Western Blotting (WB), Chromatin Immunoprecipitation (ChIP), Dot Blot (DB), ChIP DNA-Sequencing (ChIP-seq), Cleavage Under Targets and Release Using Nuclease (CUT&amp;RUN), Cleavage Under Targets and Tagmentation (CUT&amp;Tag)</td>
</tr>
</tbody>
</table>

### 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) |
| Molecular Weight | 17 kDa |
### Target Details

| 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: | Kahana, Gottschling: "DOT4 links silencing and cell growth in Saccharomyces cerevisiae." in:  
**Dot Blot**


**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 µg 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.
Validation report #104510 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

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.
Validation report #104510 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

- 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 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 CaCl₂ mix per sample (50 µl Wash Buffer + 1 µL 100 mM CaCl₂) and let it chill on ice.
Validation report #104510 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

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 0.25M (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 genomcov.
  - 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.