Datasheet for ABIN6923138

CUT&RUN Pro Set

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Overview

Quantity:	24 tests
Reactivity:	Eukaryotes
Application:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN), Cleavage Under Targets and Tagmentation (CUT&Tag)

Product Details

Purpose:	This set contains Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays, CUT&RUN Positive and Negative Control for the CUT&RUN method for improved genome-wide detection of Protein-DNA-Interactions.
Characteristics:	CUT&RUN (Cleavage Under Targets And Release Using Nuclease) offers a new approach to pursue epigenetics. CUT&RUN overcomes various downfalls of ChIP-Seq with improved workflow. CUT&RUN-Sequencing has the advantage of being a simpler technique with lower costs due to the high signal-to-noise ratio, requiring less depth in sequencing. CUT&RUN has the potential to replace all ChIP-based applications.
Components:	• CUT&RUN Positive Control (Recombinant Rabbit anti-H3K27me3 Antibody) • CUT&RUN Negative Control (Polyclonal Guinea Pig anti-Rabbit IgG Antibody, Pre-Adsorbed) • Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays
Material not included:	• Specific antibody against target of interest • pA/G-MNase (CUTANA™ pAG-MNase for ChIC/CUT&RUN Assays)

Application Details

Reagent Preparation:

- Wash Buffer
- Binding Buffer
- Antibody Buffer
- Digitonin Wash Buffer
- 2x Stop Buffer
- Low Salt Rinse Buffer
- Low Salt Incubation Buffer
- Low Salt Stop Buffer

Assay Procedure:

1. **Cell Harvest**
   - Harvest cells for each sample at RT
   - Wash cells 4 x with 1 mL Wash Buffer
2. **Prepare Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays**
   - Pipette 10 µL **Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays** slurry for each sample into a tube
   - Place the tubes on a magnet separator and remove the liquid carefully
   - Remove the tubes from the magnetic separator
   - Wash beads 3 more times with 1 mL Binding Buffer
   - Finally resuspend the beads with 10 µL Binding Buffer per sample
3. **Cell Immobilization – binding to Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays**
   - Carefully vortex the samples and add 10 µL of the prepared Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays to each sample
   - Close tubes tightly and rotate for 5-10 min at RT
4. **Cell Permeabilization and Primary Antibody Binding**
   - Place the tubes on a magnetic separator and remove the liquid carefully
   - Remove the tubes from the magnetic separator
   - Place each tube on the vortex mixer set to a low speed and add 100 µL Antibody Buffer containing Digitonin
   - Gently vortex the tubes until the beads are resuspended
   - Add 5 µL CUT&RUN anti-DYKDDDDK antibody or **CUT&RUN Positive Control** or **CUT&RUN Negative Control** corresponding to a 1:20 dilution
   - Add 1 µL primary antibody against your protein of interest corresponding to a 1:100 dilution to the remaining samples
   - Rotate the tubes for 2 h at RT or 4 h to O/N at 4 °C
   - Place the tubes on a magnet separator and remove the liquid carefully
   - Remove the tubes from the magnetic separator
   - Resuspend pellet with 1 mL Digitonin Wash Buffer and mix by inversion
   - Wash again
5. **Secondary Antibody Binding (optional)**

   If no secondary antibody is used proceed directly to pA/G-MNase-Binding.
   - Place the tubes on a magnet separator and remove the liquid carefully
   - Remove the tubes from the magnetic separator

Application Details

- Vortex the samples at low speed and add 100 µL Digitonin Wash Buffer per sample
- Add 5 µL Secondary Antibody corresponding to a 1:20 dilution
- Rotate the tubes for 1 h at 4 °C
- Place the tubes on a magnet separator and remove the liquid carefully
- Remove the tubes from the magnetic separator
- Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion
- Wash again

6. **Protein A-MNase or Protein A/G-MNase Binding**
   - Place the tubes on a magnet separator and remove the liquid carefully
   - Remove the tubes from the magnetic separator
   - Place each tube on the vortex mixer set to a low speed and add 50 µL Digitonin Wash Buffer and 2.5 µL pA/G-MNase per sample
   - Rotate the tubes for 1 h at 4 °C
   - Place the tubes on a magnet separator and remove the liquid carefully
   - Remove the tubes from the magnetic separator
   - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion
   - Wash again
7. **MNase Digestion and Release of pA/G-MNase-Bound Chromatin Fragments**

   High Ca2+/Low Salt Chromatin Cleavage
   - Quick pulse in a table-top centrifuge (max 100 x g)
   - Place the tubes on a magnet separator and remove the liquid carefully
   - Resuspend with 1 mL Low-Salt Rinse Buffer and mix by inversion
   - Quick pulse in a table-top centrifuge (max 100 x g)
   - Place the tubes on a magnet separator and remove the liquid carefully
   - Wash again
   - Place each tube on the vortex mixer set to a low speed and add 200 µL ice cold Low Salt Incubation Buffer per sample
   - Incubate tubes at 0 °C for 5 min
   - Place the tubes on a cold magnet separator and remove the liquid carefully
   - Remove the tubes from the magnetic separator
   - Resuspend with 200 µL Low Salt Stop Buffer and mix by gentle vortexing
   - Incubate tubes at 37 °C for 30 min
   - Place the tubes on a magnet separator
   - Transfer the supernatant containing the pA/G-MNase-bound digested chromatin fragments to fresh 1.5 mL tubes
   - Proceed with DNA extraction

Restrictions:	For Research Use only

Handling

Buffer:	CUT&RUN Positive Control: 50 % Glycerol/PBS, 1 % BSA, 0.09 % (w/v) Sodium Azide CUT&RUN Negative Control: 0.02 M Potassium Phosphate, 0.15 M NaCl, pH 7.2, 0.01 % (w/v)

Handling

	Sodium Azide Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays: 20 mM Sodium Acetate pH 6.6, 20 % Ethanol
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:	4 °C/-20 °C
Storage Comment:	Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays ABIN6952467 must not be frozen

Images

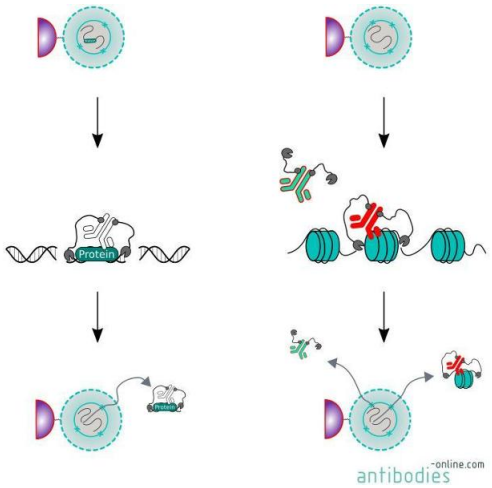


Image 1. Summary of the CUT&RUN protocol (left). Subsequently to immobilization on ConA beads (purple) and permeabilization, cells are incubated with an antibody (white) specific for the protein of interest. A Protein A-MNase or Protein A and Protein G-MNase fusion protein (grey) is then tethered to the antibody's Fc region and the MNase cleaves the DNA under the target protein. Cleavage products diffuse ouf of the cell and can be further processed for sequencing. As positive (red) and negative controls (turquois) serve antibodies that either bind to an abundant protein or that do not bind to any antigen in the cell (right).

The CUT&RUN Pro Set ABIN6923138 contains the components marked by red frames.

Validation report #104253 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

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

by New strategies to inhibit tumor angiogenesis laboratory headed by Prof. Elisabetta Dejana, IFOM - the FIRC institute of Molecular Oncology

Report Number: 104253

Date: Feb 18 2021

Target:	H3K27me3
Lot Number:	CR0109190001
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Recombinant rabbit anti-H3K27me3 antibody
Negative Control:	Polyclonal guinea pig anti-rabbit IgG antibody
Notes:	Passed. ABIN6923144 is suitable for CUT&RUN to prepare H3K27me3 targeted DNA fragments from genomic murine DNA.

Protocol:

- Cell harvest
  - Harvest 300,000 murine endothelial cells for each sample at RT. Keep cells for each sample in separate tubes.
  - Centrifuge cell solution 3 min at 600 x g at RT.
  - Remove the liquid carefully.
  - Gently resuspend cells in 1 ml Wash Buffer by pipetting and transfer cell solution to a 1.5 ml microcentrifuge tube.
  - Centrifuge cell solution 3 min at 600 x g at RT and discard the supernatant.
  - Repeat three times for a total of four washes.
  - Resuspend cell pellet for each sample in 1 ml Wash Buffer by gently pipetting.
- Concanavalin A beads preparation
  - Prepare one 1.5 ml microcentrifuge tube for each sample.
  - Gently resuspend the CUT&RUN Concanavalin A Beads.
  - Pipette 10 µl CUT&RUN Concanavalin A Beads slurry for each sample into the 1.5 ml microcentrifuge tubes.
  - Place the tubes 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 into each tube and resuspend CUT&RUN Concanavalin A Beads by gentle pipetting.
  - Spin down the liquid from the lid with a quick pulse in a bench-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 CUT&RUN Concanavalin A 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 CUT&RUN Concanavalin A Beads in Binding Buffer to each sample.
  - Close tubes tightly and rotate for 10 min at RT.
- Cell permeabilization and primary antibody binding
  - 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 100 µl Antibody Buffer containing digitonin.
  - Gently vortex the microcentrifuge tubes until the beads are resuspended.
  - Add 2 µl H3K27me3 positive control antibody ABIN6923144 corresponding to a 1:50 dilution.
  - Rotate the microcentrifuge tubes for O/N 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 clumps occur, gently remove the clumps with a 1 ml pipette tip.
  - Repeat once for a total of two washes.
- pAG-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 50 µl Digitonin Wash Buffer per sample along the side of the tube. Add 2.5µl CUTANA™ pAG-MNase for ChIC/CUT&RUN Assays (ABIN6950951, lot 19199003).
  - 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 clumps occur, gently remove the clumps with a 1 ml pipette tip.
  - Repeat once for a total of two washes.
- MNase digestion and release of pAG-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.
  - Resuspend with 1 ml Low Salt Rinse Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 ml pipette tip.
  - 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.
  - Repeat once for a total of two washes.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 200 µl ice

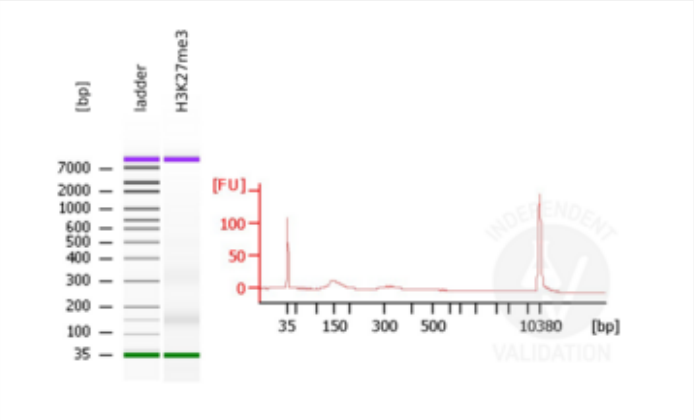
cold Low Salt Incubation Buffer per sample along the side of the tube.
    - Incubate tubes at 0 °C for 1 h.
    - Place the tubes on a cold magnet stand until the fluid is clear. Remove the liquid carefully.
    - Remove the microcentrifuge tubes from the magnetic stand.
    - Resuspend with 200 µl Low Salt Stop Solution and mix by gentle vortexing.
    - 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.
- DNA extraction
    - Add 2 µl 10% SDS to a final concentration of 0.1% and 5 µl Proteinase K (10 mg/ml) to a final concentration of 0.25 mg/ml to each supernatant containing the pAG-MNase-bound digested chromatin fragments.
    - Gently vortex tubes at a low speed of approximately 1100 rpm.
    - Incubate tubes at 37 °C O/N.
    - Add 200 µl Phenol-Chloroform-Isoamyl alcohol (PCI) to tube.
    - Vortex tubes thoroughly at high speed until the liquid appears milky.
    - Transfer liquid to a phase-lock tube.
    - Centrifuge tubes in a tabletop centrifuge at 16000 x g at RT for 5 min.
    - Carefully transfer the upper aqueous phase to a fresh 1.5 ml microcentrifuge tube containing 200 µl chloroform:isoamyl alcohol 24:1 solution.
    - Vortex tubes thoroughly at high speed until the liquid appears milky.
    - Centrifuge tubes in a benchtop centrifuge at 16000 x g at 4 °C 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 or 150 µl 5 M NH_4OAc.
    - Add 500 µl 100% ethanol.
    - Place O/N at -20 °C.
    - Centrifuge tubes in a benchtop centrifuge at 16000 x g at 4 °C for 5min.
    - Remove the liquid carefully with a pipette.
    - Add 1ml 70% ethanol.
    - Centrifuge tubes in a benchtop centrifuge at 16000 x g at 4 °C for 1 min.
    - Remove the liquid carefully with a pipette.
    - Air-dry the pellet, then dissolve in 15 µl 1 mM Tris-HCl, 0.1 mM EDTA.
- Sequencing library preparation
    - Sequencing libraries were prepared using the KAPA HyperPrep Kit (Kapa Biosystems, KR0961) according to the manufacturer's recommendations with the following modification:
    - For the post-ligation cleanup kit, the SPRI bead to ligation reaction ratio was increased to 1.1 to avoid loss of CUT&RUN products.
    - The PCR conditions were optimized for short products to avoid melting of the small fragments during elongation and favor short PCR products:
    - Initial denaturation: 1 cycle: 45 sec at 98 °C

- Amplification: 16 cycles: 15 sec at 98 °C, followed by 10 sec at 60 °C
- Final extension: 1 cycle for 1 min at 72°C

- Sample quality control
  - Evaluate DNA fragmentation via Bionanalyzer Electrophoresis before and after library preparation.

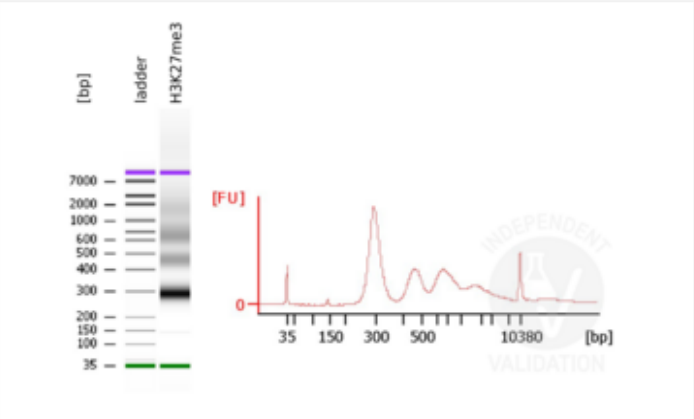
Experimental Notes:	MNase digestion was tested for 5 min, 15 min, 45 min, and 1 h. DNA from the 1 h digestion reaction was selected for library preparation because of the higher ratio of mononucleosomal fragments.

Images for Validation report #104253



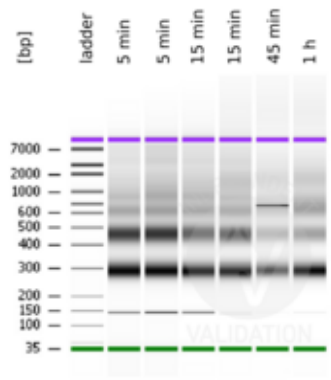
Validation image no. 1 for CUT&RUN Pro Set (ABIN6923138)

Bioanalyzer readout before library preparation using DNA fragments prepared with ABIN6923138 anti-H3K27me3 CUT&RUN positive control antibody.



Validation image no. 2 for CUT&RUN Pro Set (ABIN6923138)

Bioanalyzer readout after library preparation using DNA fragments prepared with ABIN6923138 anti-H3K27me3 CUT&RUN positive control antibody.



Validation image no. 3 for CUT&RUN Pro Set (ABIN6923138)

Bioanalyzer readout after library preparation using DNA fragments prepared with ABIN6923138 anti-H3K27me3 CUT&RUN positive control antibody and MNase digestion for the indicated times.