Datasheet for ABIN6923131

CUT&RUN Core Complete 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 CUT&RUN anti-DYKDDDDK antibody M, CUT&RUN Secondary, 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) • CUT&RUN anti-DYKDDDDK antibody M (Mouse anti-DYKDDDDK Tag Antibody) • CUT&RUN Secondary (Rabbit anti-Mouse IgG (H&L) Antibody)
Material not included:	• pA/G-MNase (CUTANA™ pAG-MNase for ChIC/CUT&RUN Assays) • CUT&RUN Concanavalin A Beads (CUT&RUN Concanavalin A Beads)

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 CUT&RUN Concanavalin A Beads**
   - Pipette 10 µL CUT&RUN Concanavalin A Beads 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 CUT&RUN Concanavalin A Beads**
   - Carefully vortex the samples and add 10 µL of the prepared CUT&RUN Concanavalin A Beads 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 M** or **CUT&RUN Positive Control** or **CUT&RUN Negative Control** corresponding to a 1:20 dilution
   - Add 1 µL **primary rabbit antibody** against your protein of interest corresponding to a 1:100 dilution to all 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
   - Vortex the samples at low speed and add 100 µL Digitonin Wash Buffer per sample
   - Add 5 µL **CUT&RUN Secondary Antibody** corresponding to a 1:20 dilution

Application Details

- 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 & CUT&RUN anti-DYKDDDDK antibody M & CUT&RUN Secondary: 0.02 M Potassium Phosphate, 0.15 M NaCl, pH 7.2, 0.01 % (w/v) Sodium Azide
Preservative:	Sodium azide

Handling

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

Images

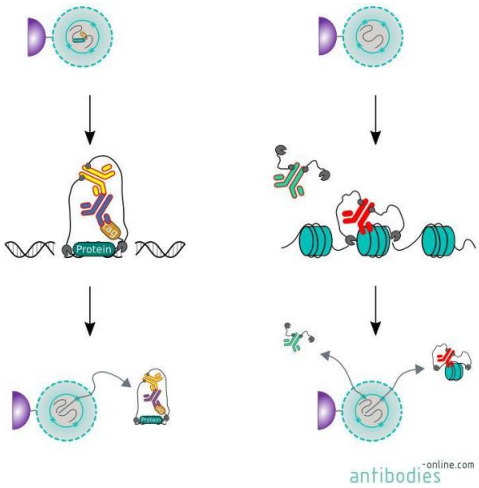

Image 1. Summary of the CUT&RUN protocol using a primary and secondary antibody (left). Subsequently to immobilization on ConA beads (purple) and permeabilization, cells are incubated with a murine antibody (blue) specific for the DYKDDDDK-tagged protein of interest and a secondary rabbit anti-mouse antibody (yellow). A Protein A-MNase or Protein A and Protein G-MNase fusion protein (grey) is then tethered to the secondary 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 Core Complete Set ABIN6923131 contains the components marked by the red frames.