Datasheet for ABIN6923144

CUT&RUN Positive Control

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Overview

Quantity:	135 µL
Application:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN), Cleavage Under Targets and Tagmentation (CUT&Tag)

Product Details

Purpose:	CUT&RUN Positive Control antibody of our CUT&RUN Sets.
Specificity:	This antibody reacts to Histone H3 trimethylated at Lysine 27 (K27me3).
Cross-Reactivity (Details):	No cross reactivity with monomethylated Lysine 27 (K27me1) or dimethylated Lysine 27 (K27me2), or other methylations in histone H3.
Characteristics:	Rabbit monoclonal to Trimethylated Histone H3 Lysine 27.
Purification:	Protein A affinity purified from an animal origin-free culture supernatant

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	0.2 µg/µL
Buffer:	50% Glycerol/PBS with 1% BSA and 0.09% 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
Expiry Date:	12 months

Publications

Product cited in:	Pagin, Pernebrink, Giubbolini, Barone, Sambruni, Zhu, Chiara, Ottolenghi, Pavesi, Wei, Cantù, Nicolis: "Sox2 controls neural stem cell self-renewal through a Fos-centered gene regulatory network." in: **Stem cells (Dayton, Ohio)**, (2021) (PubMed).

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

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Report Number: 104231

Date: Mar 01 2021

Target:	H3K27me3
Lot Number:	s-08-02482
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Recombinant anti-H3K27me3 CUT&RUN Positive Control antibody (antibodies-online, ABIN6923144)
Negative Control:	Monoclonal anti-FLAG (Sigma-Aldrich, F3165)
Notes:	Passed. ABIN6923144 serves as H3K27me3 positive control antibody in CUT&RUN.
Primary Antibody:	ABIN6923144

Protocol:

- Cell harvest
  - Harvest cells from day 10.5 mouse embryo front limbs, estimating 90,000 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 $CaCl_2$, 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.

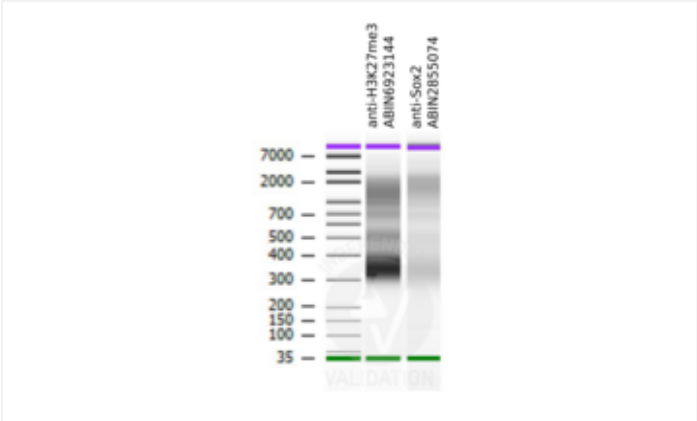
- Repeat twice for a total of three washes.
- Gently resuspend the ConA Beads in a volume of Binding Buffer corresponding to the original volume of bead slurry, i.e. 10 µL per sample.

- Cell immobilization – binding to Concanavalin A beads
  - Carefully vortex the 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 (500,000 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 rabbit anti-H3K27me3 antibody CUT&RUN positive control (antibodies-online, ABIN6923144, lot s-08-02482) 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 pA-MNase solution at 700 ng/mL (1:200 dilution of a 140 µg/mL glycerol 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

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 30 min.
- Add 100 µL 2xSTOP 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 pA-MNase-bound digested chromatin fragments to fresh 1.5 mL microcentrifuge tubes.

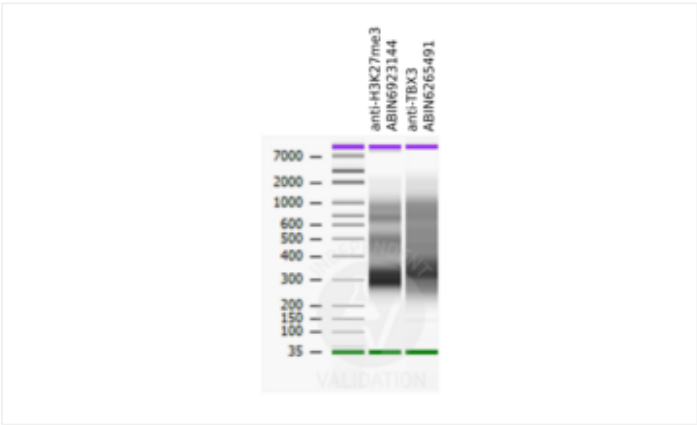
- DNA extraction
  - Add 2 µL 10% SDS to a final concentration of 0.1% and 2.5 µL Proteinase K (20 mg/mL) to each supernatant.
  - Gently vortex tubes at a low speed of approximately 1,100 rpm.
  - Incubate tubes at 50 °C for 1 h.
  - Add 200 µL PCI to tube.
  - Vortex tubes thoroughly at high speed 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 5min.
  - Remove the liquid carefully with a pipette.
  - Wash pellet with 1ml 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.
- Library preparation and sequencing
  - Libraries were prepared using KAPA HyperPrep Kit using KAPA Dual-Indexed adapters according to protocol.
  - Samples were sequenced on an Illumina NextSeq 500 sequencer, using a NextSeq 500/550 High Output Kit v2.5 (75 Cycles), 36bp PE.
- Peak calling
  - Reads were mapped to the GRCm38 (mm10) mouse genome using Bowtie2 with options: --local --very-sensitive- local --no-unal --no-mixed --no-discordant.
  - Peaks were called using MACS2 with options -f BAMPE --keep-dup all –nomodel.

Experimental Notes:	PCUT&RUN libraries generated using the H3K27me3 CUT&RUN positive control antibody ABIN6923144 show the pattern typical for a nucleosome ladder.



Validation image no. 1 for CUT&RUN Positive Control (ABIN6923144)

Bioanalyzer profiles comparing fragment size distributions between reads obtained from CUT&RUN using an anti-H3K27me3 CUT&RUN Positive Control antibody (ABIN6923144) and anti-SOX2 (ABIN2855074) after library preparation. Both samples display nucleosome size fragment distributions.



Validation image no. 2 for CUT&RUN Positive Control (ABIN6923144)

Bioanalyzer profiles comparing fragment size distributions between reads obtained from CUT&RUN using an anti-H3K27me3 CUT&RUN Positive Control antibody (ABIN6923144) and anti-TBX3 (ABIN6265491) after library preparation.