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# ChIP-seq guidelines for histone marks

## CONTENT



## Required materials

**Reagents**

iDeal ChIP-seq kit for Histones (Diagenode Cat.# C01010051)

H3K4me1 polyclonal antibody - Premium (Diagenode Cat.# C15410194)
H3K4me3 polyclonal antibody - Premium (Diagenode Cat.# C15410003)
H3K27me3 polyclonal antibody - Premium (Diagenode Cat.# C15410195)
H3K27ac polyclonal antibody - Premium (Diagenode Cat.# C15410196)
MicroChIP Diapure columns (Diagenode Cat.# C03040001)

Gloves to wear at all steps
Formaldehyde, 37%, Molecular Grade
Phosphate buffered saline (PBS) buffer
RNase/DNase-free 1.5 ml tubes
qPCR SYBR® Green Mastermix
Quant-IT dsDNA HS assay kit (Invitrogen)

**Equipment**

Diagenode Bioruptor® Pico sonication device (Cat.# B01060001)
1.5 ml Bioruptor® Microtubes with Caps (Cat.# C30010016)
IP Star® Compact Automated system (Cat.# B03000002)

Refrigerated centrifuge for 1.5 ml, 15 ml and 50 ml tubes
Rotating wheel
Vortex
Thermomixer
Qubit system (Invitrogen) qPCR cycler
Scalpel blades - Petri dishes
Tissue Lyser II (Qiagen)

## Tissue disaggregation and DNA-protein cross-linking (for frozen tissues)

1. Weigh 30-200 mg of fresh or frozen tissue in a petri dish. Keep samples on dry ice at all times and minimize the time of manipulation to prevent sample degradation.
2. Chop tissue into small pieces (between 1-3 mm3) using a scalpel blade.
3. Cool down the chopped tissue for 2 minutes in liquid nitrogen. Disaggregate the tissue using a Tissue Lyser II (Qiagen) 2 minutes at 25 strokes/sec (adapters for 2 ml tubes kept for at least 18h at -80°c).
4. Resuspend the tissue powder in 1 ml of PBS containing 1% of formaldehyde at room temperature.
5. Rotate tube for 9 min at room temperature.
6. Stop the cross-linking reaction by adding 100 µl of glycine. Continue to rotate at room temperature for 5 min.
7. Centrifuge samples at low speed (1,300 rpm) at 4°C.
8. Wash the pellet with ice-cold PBS. Aspirate the supernatant and resuspend the pellet in 1 ml of ice-cold PBS plus protease inhibitors.
9. Centrifuge at low speed (1,300 rpm) at 4°C and discard the supernatant.
10. Repeat the washing one more time.

## Cell lysis and chromatin shearing (derived from tissue samples)

11. Add 10 ml of ice-cold Lysis buffer iL1 to the pellet corresponding to 30-200 mg of tissue. Resuspend the pellet by pipetting up and down and incubate for 10 min at 4°C with gentle mixing.
12. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
13. Add 10 ml of ice-cold Lysis buffer iL2 to the pellet. Resuspend the pellet by pipetting up and down and incubate for 10 min at 4°C with gentle mixing.
14. Centrifuge for 5 min at 1300 rpm at 4°C and discard the supernatant.
15. Resuspend the pellet in 1.0 to 2.4 ml of shearing buffer iS1 containing protease inhibitors cocktail and homogenize using Tissue Lyser II (Qiagen) for 5-9 minutes at 25 strockes/sec.
16. Split the samples into 300 µl aliquots in 1.5 ml sonication tubes and incubate on ice for 10 min. Please use only recommended tubes:

    For Bioruptor® Pico, 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)
17. Shear chromatin by sonication using the Bioruptor®. An initial time course experiment is highly recommended.

    For Bioruptor® Pico, sonicate samples for 10-13 cycles (30 sec ON/30 sec OFF). Vortexing is not required between runs.
18. Transfer samples to new 1.5 ml tubes and centrifuge at 13,000 rpm for 10 min.
19. Collect the supernatant which contains the sheared chromatin.
20. Take an aliquot of 20-50 µl for assessment of chromatin shearing.

## Magnetic immunoprecipitation

21. Dilute the 5x ChIP buffer iC1 and with ChIP-seq grade water to obtain 1x ChIP buffer iC1. Place on ice.
22. Take the required amount of DiaMag Protein A-coated magnetic beads (10 µl/IP).
23. Set aside 1 µl (1%) of the sheared chromatin to use as input sample and keep at 4°C.
24. Prepare the following ChIP reaction mix (1 IP) :
    - 4 µl of 5% BSA

- 2 µl of 200x protease inhibitor cocktail
- 100 µl of 5x ChIP buffer iC1
- 100 µl of sheared chromatin. Start with the same amount of chromatin (250-1500 ng) for each condition and equilibrate with IS1 buffer.
- x µl ChIP-seq grade antibody (0.5 µg-1 µg)

25. Load the IP Star following the indirect ChIP-seq protocol (ChIP 12 hours; Beads incubation 2 hours; Washings 5 minutes).

## Elution, decross-linking and DNA purification

26. Briefly spin the tubes and place them into the Diagenode magnetic rack. Transfer the supernatant to a new tube and add 4 µl of Elution buffer iE2. Also add 100 - x µl buffer iE1 and 4 µl of buffer iE2 to the x µl input sample. Incubate for 4 hours at 65°C.
27. Purify the DNA using the Diapure columns.
28. Purified DNA was quantified using the Qubit ds DNA HS kit
29. Store ChIP DNA, and Input DNA at -20C°.

## Library preparation for sequencing

After the ChIP, library preparation is performed using the MicroPlex Library PreparationTM kit v2 (Diagenode Cat.# C05010012) protocol for each ChIP sample and for the inputs. Library preparation was performed on IP Star Compact. According to the protocol, 10-12 cycles of amplification were performed to amplify the libraries. After amplification, 1 µl of each library was loaded on Fragment Analyser to check if enough material was generated. If not, additional cycles were performed until having enough material. The additional PCR cycles were performed progressively until reaching enough amount of library for each sample.