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PROTOCOL

# Chromatin preparation from tissues for chromatin immunoprecipitation using Diagenode's Chromatin shearing optimization kit - Low SDS and Bioruptor®

## General remarks before starting

This protocol describes the chromatin preparation from fresh or frozen tissues. The isolated chromatin can be used for chromatin immunoprecipitation assays using Diagenode's iDeal ChIP-seq kit (C01010051). The following protocol is optimized for 30-40 mg of tissue allowing up to 18 ChIP samples (1.5-2 mg of tissue per sample). However, the exact amount of tissue needed for ChIP may vary depending on protein abundance, antibody affinity etc. and should be determined for each tissue type.

## Required materials and reagents

- Dounce homogenizer
- Scalpel blades
- Petri dishes
- PBS (RT and ice-cold)
- Protease inhibitor mix (Diagenode, C12010012)
- Formaldehyde (Molecular biology grade)
- Chromatin shearing optimization kit - Low SDS (Diagenode, C01020010)
- Bioruptor® (Standard, Plus or Pico) and corresponding 1.5 ml sonication tubes
- Diagenode's Elution Module (Cat. No. C01010120)
- RNase cocktail (e.g. Ambion, AM 2286 A)
- Phenol/Chloroform/Isoamyl alcohol {25:24:1}
- Chloroform/Isoamyl alcohol {24:1}
- 100% Ethanol
- 70% Ethanol

## Before starting:

Prepare the following buffers for every 30-40 mg of tissue:

- 3 ml of ice-cold PBS
- 1 ml of PBS at room temperature containing 1% of formaldehyde
- 10 ml of iL1 containing protease inhibitor mix
- 10 ml of iL2 containing protease inhibitor mix
- 1.8 ml of iS1 containing protease inhibitor mix

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## Tissue disaggregation and DNA-protein cross-linking

1. Weigh 30-40 mg of fresh or frozen tissue in a petri dish.

   *Keep samples on ice at all times and minimize the time of manipulation to prevent sample degradation.*

2. Chop tissue into small pieces (between 1-3 $mm^3$) using a scalpel blade.
3. Add 1 ml of ice-cold PBS with protease inhibitor cocktail and disaggregate the tissue using a dounce homogenizer (pestle A) to get a homogeneous suspension.
4. Transfer the tissue suspension into a 1.5 ml tube and centrifuge at 1,300 rpm for 5 min at 4°C. Gently discard the supernatant and keep the pellet.
5. Resuspend the pellet in 1 ml of PBS containing 1% of formaldehyde at room temperature.
6. Rotate tube for 8-10 min at room temperature.

   *The fixation time might require an additional optimization. Generally, histone marks require shorter fixation (8 min) than transcriptional factors (10 -15 min). Please note that stronger fixation may lead to a chromatin resistant to sonication*

7. Stop the cross-linking reaction by adding 100 µl of glycine. Continue to rotate at room temperature for 5 min.
8. Centrifuge samples at low speed (1,300 rpm) at 4°C.
9. Wash the pellet with ice-cold PBS. Aspirate the supernatant and resuspend the pellet in 1 ml of ice-cold PBS plus protease inhibitors.
10. Centrifuge at low speed (1,300 rpm) at 4°C and discard the supernatant.
11. Repeat the washing one more time.

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

12. Add 10 ml of ice-cold Lysis buffer iL1 to the pellet corresponding to 30-40 mg of tissue. Resuspend the pellet by pipetting up and down and incubate for 10 min at 4°C with gentle mixing.
13. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
14. 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.
15. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
16. Resuspend the pellet in 1.8 ml of iS1 containing protease inhibitor mix and homogenize using a dounce homogenizer (pestle B).
17. 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® Standard or Plus**, 1.5 ml TPX tubes (Diagenode, C30010009)
    **for Bioruptor® Pico**, 1.5 ml Bioruptor® Microtubes with Caps (Diagenode, C30010016)

18. Shear chromatin by sonication using the Bioruptor®. An initial time course experiment is recommended.

    **For Bioruptor® Standard or Plus** use High power setting for 10-30 cycles (30 sec ON, 30 sec OFF). Stop the system after each run of 10 cycles, vortex and spin down sample.

    **For Bioruptor® Pico**, sonicate samples for 5-15 cycles (30 sec ON, 30 sec OFF). Vortexing is not required between runs.

19. Transfer samples to new 1.5 ml tubes and centrifuge at 13,000 rpm for 10 min.

20. Collect the supernatant which contains the sheared chromatin.

21. Take an aliquot of 100 µl for assessment of chromatin shearing. The remaining chromatin can be stored at -80°C for up to 2 months for further use in the immunoprecipitation with iDeal ChIP-seq kit (C01010051)

*Note that an equivalent of at least 100.000 cells is required for the analysis of chromatin shearing.*

## Sheared chromatin analysis

This protocol refers to Diagenode's Elution Module (Cat. No. C01010120) that can be ordered separately.

Reagents not supplied:

- RNAse cocktail (e.g. Ambion, AM 2286 A)
- Phenol/Chloroform/Isoamyl alcohol {25:24:1}
- Chloroform/Isoamyl alcohol {24:1}
- 100 % Ethanol
- 70% Ethanol

1. Prepare RNase cocktail dilution (e.g. Ambion, AM 2286 A: dilute 1 µl of cocktail in 150 µl of water).
2. Add 2 µl of diluted RNase cocktail to 100 µl of chromatin sample.
3. Incubate 1h at 37°C.
4. Prepare the Complete Elution Buffer by mixing thoroughly Buffers D, E and F as follows:

| Reagents | Volume |
|---|---|
| Buffer D | 96 µl |
| Buffer E | 10 µl |
| Buffer F | 4 µl |
| Total volume | 110 µl* |

** If more than one sample is analyzed, scale accordingly*

5. Add 100 µl of the Complete Elution Buffer to each chromatin sample.
6. Mix thoroughly before incubating the sample at 65°C for 4h (or overnight).
7. Extract DNA once with an equal volume of phenol/chloroform/isoamyl alcohol {25:24:1}. Incubate the sample at RT for 10 min on a rotating wheel.
8. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
9. Add 1 volume of chloroform/isoamyl alcohol {24:1}. Incubate the sample at RT for 10 min on a rotating wheel.
10. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
11. Precipitate the DNA by adding 5 µl of meDNA IP coprecipitant, 10 µl of meDNA-IP precipitant and 500 µl of cold 100% ethanol to the sample. Incubate at -80 °C for 30 min.
12. Centrifuge for 25 min at 13,000 rpm at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70% ethanol to the pellet.

13. Centrifuge for 10 min at 13,000 rpm at 4°C. Carefully remove the supernatant, leave tubes open for 30 min at RT to evaporate the remaining ethanol.
14. Re-suspended the pellet in 20 µl of TE buffer.
15. Run samples (20 µl of DNA + 4 µl of 6x loading dye) on a 1.5% agarose gel.

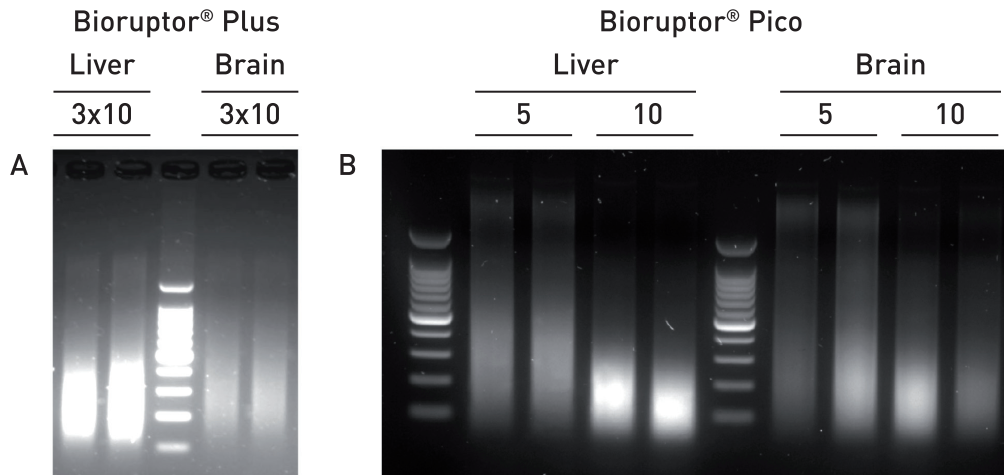


**Figure 1. Chromatin shearing efficiency from mouse liver and brain using Diagenode's Chromatin Shearing Optimization kit – Low SDS** (C01020010). Panel A: Chromatin from mouse liver and brain was sheared using the Bioruptor® Plus for 3x10 cycles (30'' On/30'' Off). Panel B: Chromatin from mouse liver and brain was sheared using the Bioruptor® Pico for 5 and 10 cycles (30'' On/30'' Off). 100 bp ladder is loaded as size standard.

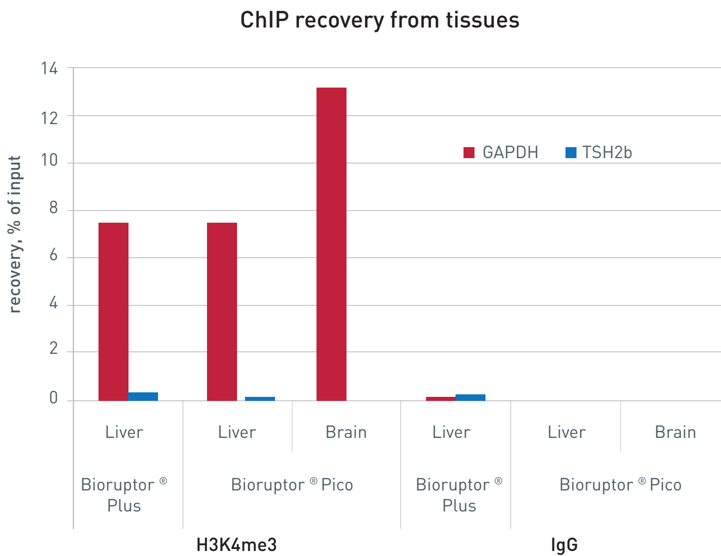


**Figure 2: Chromatin immunoprecipitation from mouse liver and brain.**

Chromatin was prepared from mouse liver and brain by sonication on Bioruptor® Plus (3x10 cycles) or Bioruptor® Pico (10 cycles). Diagenode's H3K4m3 antibody (C15410003) or negarive IgG control (C15410206) were used for chromatin immunoprecipitation. Quantitative PCR was performed with the positive control mouse GAPDH and the negative control mouse TSH2B primer sets. The recovery is expressed as % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

PRO_Chr_prep-from tissue-for-chr-immuno_05_14