diagenode

Innovating Epigenetic Solutions

# Chromatin shearing optimization kit

## Low SDS

(for Histones)

Cat. No. **C01020010**

**Version 4** | 04.16

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# Content

MANUAL

# Introduction

The first critical step of a successful Chromatin Immunoprecipitation (ChIP) experiment is the preparation of sheared chromatin. We therefore suggest the use one of our optimized Shearing ChIP kits.

Chromatin Shearing Optimization kit - **Low** SDS (for Histones) (Cat. No.C01020010)

Chromatin Shearing Optimization kit – **Low** SDS (for Transcription Factors) (Cat. No. C01020013)

Chromatin Shearing Optimization kit - **Medium** SDS (Cat. No.C01020011)

Chromatin Shearing Optimization kit - **High** SDS (Cat. No.C01020012)

Our Chromatin Shearing Optimization kits are used in combination with the Bioruptor® in order to ensure a highly reproducible chromatin shearing and make sure you obtain the right fragment size needed for your experiment. Establish optimal conditions required for shearing cross-linked chromatin (protein-DNA) is usually laborious; the protocol of the Chromatin Shearing Optimization kits is fast, easy-to-use and optimized to get the best results possible.

The ChIP assay offers great potential to improve knowledge about the regulation of gene expression. This technique is now used in a variety of life science disciplines including cellular differentiation, tumor suppressor gene silencing, and the effect of histone modifications on gene expression.

Isolation and shearing of the chromatin after formaldehyde fixation is the first step in the ChIP method. The following two steps are: 1/ specific immunoprecipitation of chromatin fragments using antibody against the protein of interest and 2/ analysis of the immunoprecipitated DNA fragment. With the present kit, the first step of the ChIP method is guaranteed, providing you with the best results.

Using standard protocols and kits leads to a significant decrease in potential variables that can occur from shearing to ChIP, which makes interpretation and analysis from experiment to experiment much more accessible.

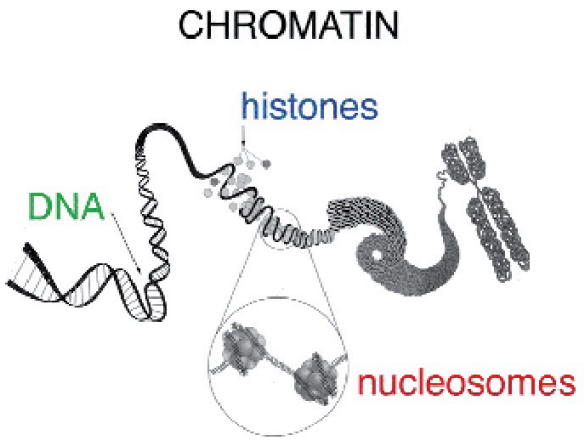


**Figure 1**

**Representation of the chromatin**

Chromatin is that portion of the cell nucleus which contains all the DNA of the nucleus in animal or plant cells. DNA in chromatin is organised in arrays of nucleosomes. Two copies of histone proteins are assembled into an octamer that has 145-147 base pairs (bp) of DNA wrapped around it to form a nucleosome core. The nucleosome, in its role as the principal packaging element of DNA within the nucleus, is the primary determinant of DNA accessibility.

(Luger et al. 1997)

# Kit method overview & time table

**Table 1 : Protocol overview**

| Step | | Time needed |
|---|---|---|
| 1 | Cell collection and DNA-protein crosslinking | 1 to 2 hours |
| 2 | Cell lysis and Chromatin shearing | 1 to 2 hours |
| 3 | Sheared chromatin analysis | 1 day |

# Kit Content

**Table 2: Kit content and storage**

This kit contains enough reagents for the shearing of chromatin from 100 million cells. These components are identical to the ones included in the iDeal ChIP-seq kit (C01010050, C01010051 and C01010059).

| Description | quantity | storage |
|---|---|---|
| **Glycine** | 580 µl | 4°C |
| **Lysis Buffer iL1** | 110 ml | 4°C |
| **Lysis Buffer iL2** | 110 ml | 4°C |
| **Shearing Buffer iS1** | 11 ml | 4°C |
| **Elution Buffer iE1** | 5 ml | 4°C |
| **Elution Buffer iE2** | 400 µl | 4°C |
| **TE buffer** | 2 ml | 4°C |
| **DNA precipitant** | 1 ml | 4°C |
| **DNA co-precipitant** | 500 µl | -20°C |
| **Protease inhibitor cocktail** | 50 µl | -20°C |

# Required Materials Not Provided

## Reagents

- Formaldehyde (37% stock)
- Ice-cold PBS buffer
- Agarose and TAE buffer
- DNA molecular weight marker
- RNase cocktail (e.g. Ambion, AM 2286 A)
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)

- 100% Ethanol
- 70% Ethanol

### Equipment

- Cell scraper
- Centrifuges (at 4°C) for 1.5 ml tubes and 15 ml tubes
- Bioruptor® from Diagenode (with recommended consumables)
- Agarose gel apparatus
- 30°C incubator or water bath

### (for tissues)

- Dounce homogenizer with loose and tight pestiles (2 mL)
- Scalpel blades
- Petri dishes

# Remarks before starting

Before starting the ChIP, the chromatin should be sheared to fragments in the 100 to 600 bp range. Our kits and protocols are optimized for Chromatin shearing using the Bioruptor® (Pico, Plus and Standard). The maximum volume for shearing with the Bioruptor® is 300 µl per 1.5 ml microtube (depending on the specific type). We recommend using TPX tubes (Cat. No.C30010010-1000) for Bioruptor® Plus and Standard and 1.5 ml Bioruptor® Microtubes with caps (C30010016) for Bioruptor® Pico as shearing has been shown to be more efficient and reproducible using these tubes. The shearing conditions mentioned in the protocol are adequate for a variety of cell types

However, given that cell types are different, we recommend optimizing sonication conditions for each cell type before processing large quantities of cells or samples. Perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation.

# Short protocol for experienced users

## STEP 1a. Cell fixation and collection

1. Collect the cells by trypsinisation and wash two times with PBS.
2. Count the cells and resuspend them in PBS to obtain up to 10 million cells in 500 µl of PBS. Aliquot 500 µl of cell suspension in 1.5 ml tubes.
3. Add 13.5 µl of formaldehyde 37%. Mix by gentle vortexing and incubate for 8 minutes at room temperature to allow fixation to take place.
4. Stop the fixation by adding 57 µl of **Glycine** solution. Mix by gentle vortexing and incubate for 5 minutes at room temperature. Work on ice from this point onwards.
5. Centrifuge at 1,600 rpm (500 x g) for 5 minutes at 4°C and gently aspirate the supernatant without disturbing the cell pellet.
6. Wash the cells twice with 1 ml PBS.

## STEP 1b. Tissue disaggregation and DNA-protein cross-linking (derived from fresh or snap-frozen tissue samples)

7. 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.*
8. Chop tissue into small pieces (between 1-3 mm3) using a scalpel blade.
9. Add 1 ml of ice-cold PBS with protease inhibitor cocktail and disaggregate the tissue using a dounce homogenizer (loose pestle) to get a homogeneous suspension.
10. 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.
11. Resuspend the pellet in 1 ml of PBS containing 1% of formaldehyde at room temperature.
12. 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*
13. Stop the cross-linking reaction by adding 100 µl of glycine. Continue to rotate at room temperature for 5 min.
14. Centrifuge samples at low speed (1,300 rpm) at 4°C.
15. Wash the pellet with ice-cold PBS. Aspirate the supernatant and resuspend the pellet in 1 ml of ice-cold PBS plus protease inhibitors.
16. Centrifuge at low speed (1,300 rpm) at 4°C and discard the supernatant.
17. Repeat the washing one more time.

## STEP 2a. Cell lysis and chromatin shearing from cells.

18. Add 10 ml of ice-cold **Lysis buffer iL1** to the cell pellet corresponding to 10 million cells. Resuspend the cells by pipetting up and down several times and incubate for 10 minutes at 4°C with gentle mixing. Scale down accordingly when using fewer cells.
19. Centrifuge for 5 minutes at 1,600 rpm (500 x g) and 4°C and discard the supernatant.

20. Add 10 ml of ice-cold **Lysis buffer iL2** to the cell pellet. Resuspend the cells by pipetting up and down several times and incubate for 10 minutes at 4°C with gentle mixing.
21. Centrifuge for 5 minutes at 1,600 rpm (500 x g) and 4°C and discard the supernatant.
22. Add 200x **protease inhibitor cocktail** to the **Shearing buffer iS1**. Keep on ice.
23. Add 1 ml of **Shearing buffer iS1** containing protease inhibitor to 10 million cells. Resuspend by pipetting up and down and incubate on ice for 10 minutes.
24. 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 seconds ON, 30 seconds OFF). Stop the system after each run of 10 cycles, vortex and spin down sample.
    - **For Bioruptor® Pico**, sonicate samples for 5-15cycles (30 seconds ON, 30 seconds OFF). Vortexing is not required between runs.Centrifuge at 13,000 rpm (16,000 x g) for 10 minutes and collect the supernatant which contains the sheared chromatin
25. Centrifuge at 13,000 rpm (16,000 x g) for 10 minutes and collect the supernatant which contains the sheared chromatin

## STEP 2b. Cell lysis, and chromatin shearing (derived from tissue sample)

26. Add 10 ml of ice-cold Lysis buffer iL1 to the pellet corresponding to 30-40 mg of tissue. Resuspend the pellet by pippeting up and down and incubate for 10 min at 4°C with gentle mixing.
27. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
28. Add 10 ml of ice-cold Lysis buffer iL2 to the pellet. Resuspend the pellet by pippeting up and down and incubate for 10 min at 4°C with gentle mixing.
29. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
30. Resuspend the pellet in 1.8 ml of iS1 containing protease inhibitor mix and homogenize using a dounce homogenizer (tight pestle).
31. 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)
32. 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.
33. Transfer samples to new 1.5 ml tubes and centrifuge at 13,000 rpm for 10 min.
34. Collect the supernatant which contains the sheared chromatin.
35. 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.*

## STEP 3. Sheared chromatin analysis

36. Spin 50 µl of the sheared chromatin at 12,000 rpm for 10 min at 4°C. Use the supernatant for chromatin analysis.
37. Add 2 µl of diluted RNase cocktail and incubate 1h at 37°C.

38. Add 50 µl of elution buffer iE1 and 4 µl of elution buffer iE2, mix thoroughly.
39. Incubate samples at 65°C for 4h (or overnight).
40. 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.
41. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
42. Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the sample at RT for 10 min on a rotating wheel.
43. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
44. Precipitate the DNA by adding 10 µl DNA precipitant, 5 µl of co-precipitant, and 500 µl of cold 100% ethanol to the sample. Incubate at -80 °C for 30 min.
45. Centrifuge for 25 min at 13,000rpm at 4°C. Add 500 µl of ice-cold 70% ethanol to the pellet.
46. Centrifuge for 10 min at 13,000 rpm at 4°C. Air-dry the pellet.
47. Re-suspended the pellet in 20 µl of TE buffer.
48. Run samples in a 1.5% agarose gel.

# Detailed protocol

## STEP 1a. Cell fixation and collection

The protocol below is intended for adherent cells. Collect suspension cells by centrifugation and continue with the protocol starting from step 6.

1. Pre-warm PBS, culture medium and trypsin-EDTA at 37°C.
2. Remove the medium and rinse the cells with pre-warmed PBS (10 ml for a 75 cm2 culture flask). Gently shake the flask for 2 minutes.
3. Remove the PBS and add sterile trypsin-EDTA to the culture flask to detach adherent cells from the bottom. Table 3 shows the required amount of trypsin for different numbers of cells. Gently shake the culture flask for 1-2 minutes or until the cells start to detach. The time needed may depend on the cell type but do not continue trypsin treatment longer then necessary as prolonged treatment with trypsin may damage the cells. Regularly check if the cells start to detach.

| Table 3 | | | |
|---|---|---|---|
| # of cells | 3x 10e6 cells | 10e7 cells | 5x 10e7 cells |
| Trypsin-EDTA | 1 ml | 3 ml | 15 ml |

4. Immediately add fresh culture medium to the cells when they are detached (Table 4). This will inactivate trypsin. Tansfer cell suspension to a 50 ml tube.

| Table 4 | | | |
|---|---|---|---|
| # of cells | 3x 10e6 cells | 10e7 cells | 5x 10e7 cells |
| Culture medium | 2 ml | 6 ml | 30 ml |

5. Rinse the flask by adding 10 ml of PBS. Add this volume to your 50 ml tubes containing cells from point 4.

6. Centrifuge for 5 minutes at 1600 rpm and 4°C and remove the supernatant.
7. Resuspend the cells in 20 ml of PBS and count them. Collect the cells by centrifugation for 5 minutes at 1600 rpm and 4°C.
8. Resuspend the cells in PBS to obtain a concentration of up to 10 million cells per 500 µl of PBS. If desired, the cell concentration can be decreased down to 1 million per 500 µl. Label 1.5 ml tubes and aliquot 500 µl of cell suspension in each tube.
9. Add 13.5 µl of formaldehyde 37% to each tube containing 500 µl of cell suspension. Mix by gentle vortexing and incubate for 8 minutes at room temperature to allow fixation to take place.
10. Add 57 µl of Glycine to the cells to stop the fixation. Mix by gentle vortexing and incubate for 5 minutes at room temperature. Keep the cells on ice from this point onwards.
11. Collect the cells by centrifugation at 1600 rpm for 5 minutes and 4°C. Discard the supernatant without disturbing the cell pellet.
12. Wash the cells twice with 1 ml of cold PBS.

## STEP 1b. Tissue disaggregation and DNA-protein cross-linking (derived from fresh or snap-frozen tissue samples)

13. 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.*
14. Chop tissue into small pieces (between 1-3 mm3) using a scalpel blade.
15. Add 1 ml of ice-cold PBS with protease inhibitor cocktail and disaggregate the tissue using a dounce homogenizer (loose pestle) to get a homogeneous suspension.
16. 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.
17. Resuspend the pellet in 1 ml of PBS containing 1% of formaldehyde at room temperature.
18. 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*
19. Stop the cross-linking reaction by adding 100 µl of glycine. Continue to rotate at room temperature for 5 min.
20. Centrifuge samples at low speed (1,300 rpm) at 4°C.
21. Wash the pellet with ice-cold PBS. Aspirate the supernatant and resuspend the pellet in 1 ml of ice-cold PBS plus protease inhibitors.
22. Centrifuge at low speed (1,300 rpm) at 4°C and discard the supernatant.
23. Repeat the washing one more time.

## STEP 2a. Cell lysis and chromatin shearing from cells

24. Add 1 ml of ice-cold **Lysis buffer iL1** to the 1.5 ml tube containing 10 million cells. Resuspend the cells by pipetting up and down several times and transfer them to a 15 ml tube. Add 9 ml of **buffer iL1** and incubate for 10 minutes at 4°C with gentle mixing. If the starting amount of cells was less then 10 million, scale down accordingly (e.g. use a total of 5 ml **buffer iL1** for 5 million cells).

25. Pellet the cells by centrifugation at 1,600 rpm for 5 minutes and 4°C and discard the supernatant.
26. Add 1 ml of ice-cold **Lysis buffer iL2** and resuspend the cells by pipetting up and down several times. Add another 9 ml of **buffer iL2** and incubate for 10 minutes at 4°C with gentle mixing. Scale down accordingly when using less then 10 million cells.
27. Pellet the cells again by centrifugation for 5 minutes at 1,600 rpm (500 x g) and 4°C and discard supernatant.
28. Add 200x **protease inhibitor cocktail** to **Shearing buffer iS1**. Prepare 1 ml of complete shearing buffer per tube of 10 million cells. Keep on ice.
29. Add 1 ml of complete **Shearing buffer iS1** to 10 million cells. Resuspend the cells by pipetting up and down several times. The final cell concentration should be 1 million cells per 100 µl **buffer iS1**. Split into aliquots of 100 to 300 µl and transfer the cell suspension to 1.5 ml TPX microtubes (Cat. No.C30010010-1000 ) when using the Bioruptor Standard or Plus or to 1.5 ml Bioruptor® Microtubes with Caps (Cat No C30010016) optimized for chromatin shearing with the Bioruptor® Pico.
30. Shear the chromatin by sonication using the Bioruptor®. When using the Bioruptor Standard or Plus, shear for 1 to 3 runs of 10 cycles [30 seconds "ON", 30 seconds "OFF"] each at high power setting. Briefly vortex and spin between each run. Shear for 8-10 cycles [30 seconds "ON", 30 seconds "OFF"] when using the Bioruptor Pico. These shearing conditions will work excellent for many cell types. However, depending on the cell type and Bioruptor® system used, optimisation may be required.
31. Centrifuge at 13,000 rpm (16,000 x g) for 10 minutes and collect the supernatant which contains the sheared chromatin. Use 50 µl of the sheared chromatin for analysis of shearing efficiency (step 3). The remaining chromatin can be used immidiately in immunoprecipitation of stored at -80°C for up to 2 months.

## STEP 2b. Cell lysis, and chromatin shearing (derived from tissue sample)

32. Add 10 ml of ice-cold Lysis buffer iL1 to the pellet corresponding to 30-40 mg of tissue. Resuspend the pellet by pippeting up and down and incubate for 10 min at 4°C with gentle mixing.
33. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
34. Add 10 ml of ice-cold Lysis buffer iL2 to the pellet. Resuspend the pellet by pippeting up and down and incubate for 10 min at 4°C with gentle mixing.
35. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
36. Resuspend the pellet in 1.8 ml of iS1 containing protease inhibitor mix and homogenize using a dounce homogenizer (tight pestle).
37. 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)
38. 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.
39. Transfer samples to new 1.5 ml tubes and centrifuge at 13,000 rpm for 10 min.
40. Collect the supernatant which contains the sheared chromatin.
41. 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.*

## STEP 3. Sheared chromatin analysis

**42.** Take an aliquot of 50 µl of sheared chromatin and spin the chromatin at 12,000 rpm for 10 min at 4°C. Transfer the supernatant to a new tube for chromatin analysis.

**43.** Prepare RNase cocktail dilution (e.g. Ambion, AM 2286 A: dilute 1µl of cocktail in 150 µl of ChIP-seq grade water).

**44.** Add 2 µl of diluted RNase cocktail.

**45.** Incubate 1h at 37°C.

**46.** Add 50 µl of elution buffer iE1.

**47.** Add 4 µl of elution buffer iE2, mix thoroughly.

**48.** Incubate samples at 65°C for 4h (or overnight).

**49.** 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.

**50.** Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

**51.** Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the sample at RT for 10 min on a rotating wheel.

**52.** Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

**53.** Precipitate the DNA by adding 10 µl DNA precipitant, 5 µl of co-precipitant, and 500 µl of cold 100% ethanol to the sample. Incubate at -80 °C for 30 min.

**54.** Centrifuge for 25 min at 13,000rpm at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70% ethanol to the pellet.

**55.** 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.

**56.** Re-suspended the pellet in 20 µl of TE buffer.

**57.** Run samples (20 µl of DNA + 4 µl of 6x loading dye) in a 1.5% agarose gel.

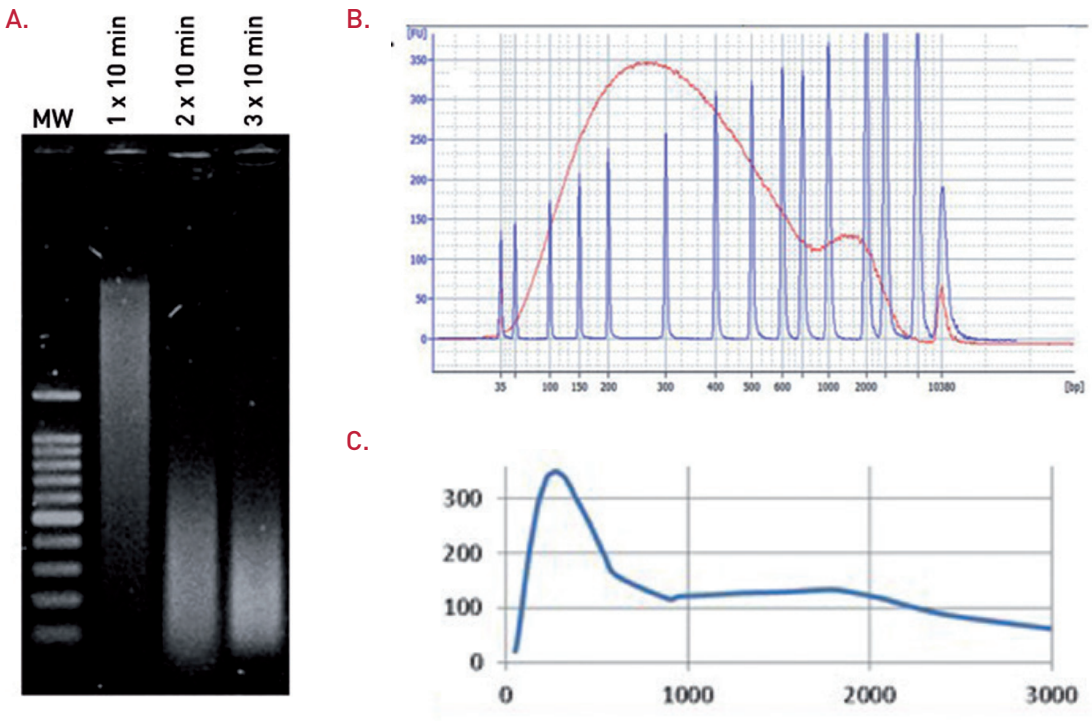


**Figure 2**

**Successful Chromatin shearing of chromatin from «difficult cells» (K562)**

K562 cells (myelogenous leukemia cell line) are fixed with 1% formaldehyde (for 8 min at RT). Nuclei isolation of 1 million fresh cells are performed using buffers of Diagenode's Chromatin Shearing Optimization kit - Low SDS (Cat. No. AA-001-0100) and are then resuspended in 100 µl of Shearing Buffer iS1 prior to Chromatin shearing.

Samples are sheared during 1, 2 or 3 rounds of 10 cycles of 30 sec ON/ 30 sec OFF with the Bioruptor® PLUS combined with the Bioruptor® Water cooler (Cat. No. BioAcc-Cool) & Single Cycle Valve (Cat. No. VB-100-0001) at HIGH power setting (position H). For optimal results, samples are vortexed before and after performing 10 sonication cycles, followed by a short centrifugation at 4°C. All samples were treated with RNase and reverse cross-linked.

**Panel A:** 10 µl of DNA (equivalent to 300 ng) are analyzed on a 1.5% agarose gel.

**Panel B and C**: Third sample of panel A (3x 10 min) was analyzed on Bioanalyzer 2100 using DNA High Sensitivity chip. The default log scaled Bioanalyzer output can be seen in Panel A, while Panel C represents their linear transformation for better vizualisation. Out of range fragments were not shown on this graph.

This example clearly shows that «difficult cells» such as K562 can readily be sheared using the Bioruptor®.

# Troubleshooting guide

| Process | Protocol step | Issues and resolutions |
|---|---|---|
| Crosslinking and fixation | Optimize crosslinking time | Poor crosslinking causes DNA loss, elevated background, and/or reduced antigen availability in chromatin. Emperically determine optimal crosslinking time for maximal specificity and efficiency of ChIP. The optimal duration of cross-linking varies between cell type and protein of interest. Short crosslinking time (5-10 minutes) may improve shearing efficiency. Crosslinking duration should not exceed 30 minutes or shearing will be inefficient. |
| | Assure proper fixation time with formaldehyde | Crosslinking may be too weak or too strong without proper fixation time. Optimize fixation step e.g: incubate for 8 minutes at room temperature with high-quality, fresh 1% formaldehyde final concentration (weight/volume). |
| | Optimize formaldehyde concentration | Lower formaldehyde concentrations (1%weight/volume) may improve shearing efficiency. For some proteins, however, especially those that do not directly bind DNA, this might reduce crosslinking efficiency and thus the yield of precipitated chromatin. Empirically determine the formaldehyde concentration as some antigen epitopes may be more sensitive to formaldehyde. |
| Cell lysis | Make sure cells disrupt completely | Do not use too many cells per amount of lysis buffer (w/v) so that cells can be completely disrupted. Follow the instructions in the protocol (e.g.: 1 million cells or less/130 µl of complete Buffer B). See steps 2 and 3. |
| | Maintain cold temperature during lysis | Perform cell lysis at 4°C (cold room) or on ice. Always keep the samples ice cold during cell lysis and use cold buffers as in Step 3. |
| | Prevent protein degradation | Add the protease inhibitors to the lysis buffer immediately before use. |
| Cell type | Determine which cell types have previously been validated with the kit | HeLa, NCCIT 293T, Chondrocytes, P19, ASC (adipose stem cells), U2OS and kerationocytes have been used to validate this magnetic ChIP protocol. |
| Number of cells required | Determine number of cells for ChIP | The number of cells for ChIP is determined by cell type, protein of interest, and antibodies used. Use chromatin from 1,000 to 10,000 cells per ChIP. (In some cases, chromatin from up to 100,000 cells may be needed). You may need to empirically determine the optimal number. |
| Chromatin shearing | Maintain 4°C temperature during shearing | Keep samples cold at 4°C before sonication to maintain sample integrity. |
| | Maintain 0ÐC temperature during sonication | Maintain temperature of the samples at 4°C to maintain sample integrity. |
| | Optimize SDS concentration | High % SDS favours better sonication but inhibits immunoselection (optimal range: 0.1% to 1%). Final SDS concentration should not be higher than 0.15 to 0.20% (e.g. If the shearing buffer contains 0.75% SDS, the sheared chromatin is diluted 3.5 to 4.0 fold in the ChIP buffer. |
| | Determine amount of sheared chromatin needed for ChIP | Most of the sheared chromatin will be used for ChIP and the input control. A small amount will be checked on agarose gel. |
| | Dilute the sheared chromatin in ChIP buffer for Immunoselection incubation | The sheared chromatin is diluted in complete Buffer A prior to the immunoselection incubation (see Step 3). Dilute the sheared chromatin at least 7-fold. Adjust the ChIP buffer volume added to the chromatin accordingly. |

| | | |
|---|---|---|
| Sonication tips | Determine cell number | Start with 1x10e6 cells or less. |
| | Sonication conditions with the Bioruptor® | Shear the samples of chromatin using the Bioruptor® for 10-12 cycles of: [30 seconds "ON" / 30 seconds "OFF"] each. These conditions were tested with many mammalian cell lines and were excellent for subsequent ChIP experiments. A troubleshooting guide for Bioruptor-chromatin shearing is available. |
| | Chromatin shearing with Diagenode modules | You can also use the other Chromatin Shearing Optimization kits which differ by their SDS concentration (Cat. No. AA-001-0100 ; AA-002-0100) |
| Gel analysis of sheared chromatin | Load enough DNA on gel | Chromatin equivalent to at least 100,000 cells can be be visualized on a gel. Do not use an excessive amount or it will obscure the visualization. The DNA amount to load depends on well size and on the gel size. |
| | Use correct agarose concentration | Use a 1-1.5% agarose gel. |
| | Use correct running buffer concentration and run time | 1x TAE or TBE is preferred to 0.5x TAE (which can lead to smears). Run slowly. |

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