diagenode

A Hologic Company

Chromatin EasyShear Kit - High SDS

Previous name: Chromatin Shearing Optimization Kit – High SDS (True MicroChIP kit)

Cat. No. C01020012

Compatible with:

True MicroChIP Kit

µChIPmentation Kit for Histones

USER GUIDE

Version 6 03_2021

Please read this manual carefully
before starting your experiment

Contents

Introduction

Chromatin shearing is a crucial step for the success of ChIP experiments. It is important to establish optimal conditions to shear cross-linked chromatin to get the correct fragment sizes needed for ChIP. A successful chromatin preparation relies on the optimization of **cross-linking**, **cell lysis** and **sonication** itself. All 3 steps are interconnected and need to be optimized individually for different experimental settings. Our **Chromatin EasyShear Kits (previous name: Chromatin Shearing Optimization Kits)** together with the **Bioruptor®** combine **efficient cell lysis** and **chromatin shearing** leading to **consistent results**.

The Chromatin EasyShear Kits are recommended for:

- The optimization of the chromatin shearing of a new cell line/ new sample type prior to ChIP using Diagenode's ChIP kits
- The optimization of the chromatin shearing and/or chromatin preparation prior to ChIP using other protocols

Each Chromatin EasyShear Kit provides optimized reagents and a thoroughly validated protocol according to your specific experimental needs. SDS concentration is adapted to each workflow taking into account target-specific requirements.

Choose an appropriate kit for your specific experimental needs and get consistent results.

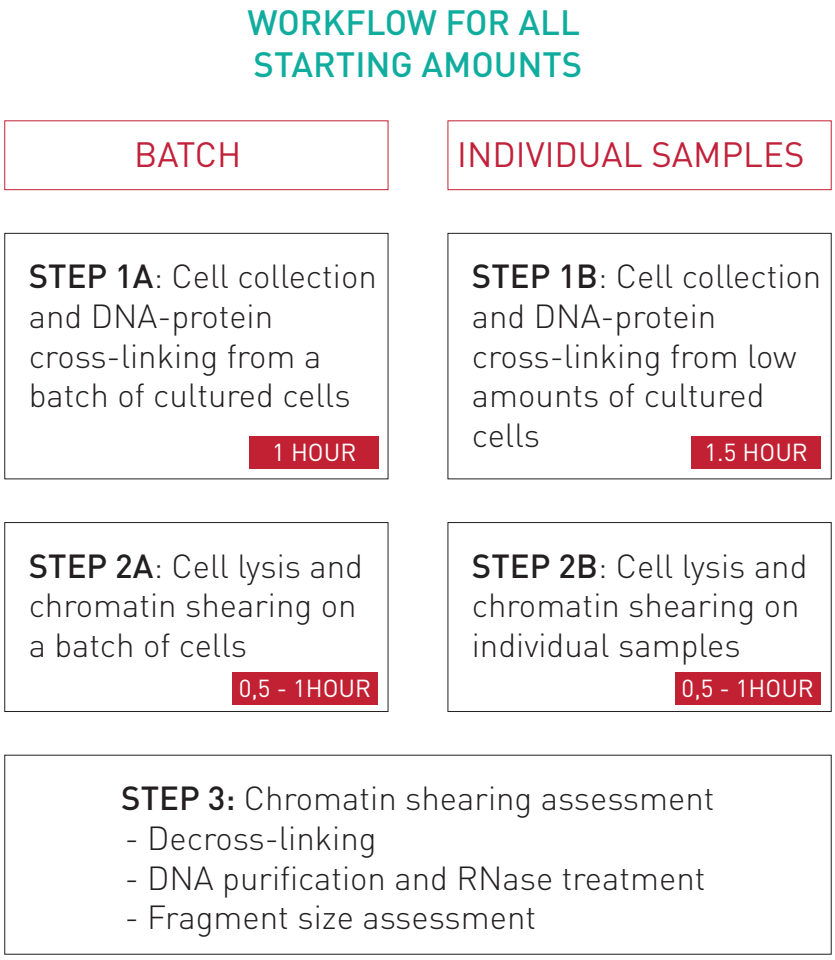
	Chromatin EasyShear Kit Ultra Low SDS	**Chromatin EasyShear Kit** Low SDS	**Chromatin EasyShear Kit** for Plant	**Chromatin EasyShear Kit** High SDS
Cat. No.	C01020010	C01020013	C01020014	C01020012
Sample type	Cells, tissue	Cells, tissue	Plant tissue	Cells - low amount
Target	Histones	Transcription Factors and histones	Histones	Histones
Nuclei isolation	Yes	Yes	Yes	No
SDS concentration	< 0.1%	0.2%	0.5%	1%
Corresponding to shearing buffers from	iDeal ChIP-seq Kit for Histones ChIPmentation Kit for Histones	iDeal ChIP-seq Kit for Transcription Factors iDeal ChIP qPCR Kit iDeal ChIP-FFPE Kit	Universal Plant ChIP-seq Kit	True MicroChIP Kit µChIPmentation for Histones

Kit Method Overview

The Chromatin EasyShear Kit – High SDS is validated for the chromatin preparation from a **limited number of cells** and can be used for the optimization of chromatin preparation prior to ChIP on histones performed with the following Diagenode kits:

ChIP kit	Manual version	Automated version
TrueMicro ChIP kit	C01010130	C01010140
µChIPmentation Kit for Histones	C01011011 C01011012	N/A

If non-Diagenode protocol will be used for immunoprecipitation step, sheared chromatin should be diluted at least 5 times per immunoprecipitation reaction with a detergent-free buffer (no SDS, sodium deoxycholate etc...).



Kit materials

The Chromatin EasyShear Kit – High SDS allows a nice flexibility in the cell number and experimental design. It contains enough reagents to perform 150 chromatin preparations in most of the experimental plans.

This kit does not contain reagents for DNA purification. We highly recommend using MicroChIP DiaPure columns (Diagenode, Cat. No. C03040001), which can be purchased separately.

The kit content is described in Table 2. Upon receipt, store the components at the indicated temperatures.

Table 2. Components supplied with the Chromatin EasyShear Kit - High SDS

Description	Quantity	Storage
Glycine	17.25 ml	4°C
Lysis Buffer tL1	3.75 ml	4°C
Elution Buffer tE1	15 ml	4°C
Elution Buffer tE2	1200 µl	4°C
TE Buffer	7.5 ml	4°C
Protease inhibitors cocktail	160 µl	-20°C

Required materials not provided

Materials and Reagents

- Gloves to wear at all steps
- RNase/DNase-free 1.5 and 15 ml tubes
- Formaldehyde, 37%, Molecular Grade
- Phosphate buffered saline (PBS)
- Cell culture medium
- Hank's balanced salt solution (HBSS – e.g. ThermoFisher Scientific, 14175095)
- MicroChIP DiaPure columns (Diagenode, Cat. No. C03040001)
- RNase cocktail (e.g. Ambion, AM2286)

Equipment

- Fume hood
- Bioruptor® sonication device and the associated microtubes:
  - **Bioruptor Pico (Diagenode, Cat. No. B01060010)**,
  - 0.2 ml microtubes (Cat. No. C30010020) or
  - 0.65ml microtubes (Cat. No. C30010011) or
  - 1.5 ml microtubes with caps (Cat. No. C30010016)
  - **Bioruptor Plus (Diagenode, Cat. No. B01020003)**,
  - 0.5ml microtubes (Cat. No. C30010013) or
  - 1.5 ml TPX microtubes (Cat. No. C30010010-300)
- Refrigerated centrifuge for 1.5 ml tubes
- Centrifuge for 15 ml tubes
- Thermomixer
- Cell counter
- Vortex
- DNA sizing equipment (an automated capillary electrophoresis instrument, e.g. Fragment Analyzer or Bioanalyzer (Agilent) and High Sensitivity Kits or agarose gel electrophoresis)

Remarks before starting

A high-quality chromatin sample combines two main features: a suitable fragment size range and the availability of cross-linked epitopes for ChIP. A perfect sonication profile is a profile from which the highest specific signal and the lowest background are generated.

There is not one universal size range generally applicable for ChIP. As a rule, fragments between **100-600 bp** are suitable for the majority of ChIP experiments and can be used as a starting point. This range can be fine-tuned for particular experimental requirements depending on the specificity of the target (histones or non-histone proteins) and the required down-stream analysis (ChIP-qPCR or ChIP-seq). Generally, a tighter fragment distribution is recommended for ChIP-seq than for ChIP-qPCR. Note that **100-300 bp** is compatible (but not absolutely necessary) with **histone ChIP-seq** while a **broader fragment range** is more suitable for **non-histone ChIP-seq** (transcriptional factors and proteins that are not bound directly to DNA and for long-distance interacting proteins). With the recent evolution of sequencing technologies, it is common to perform ChIP-seq experiments from chromatin with a distribution in the 100–800 bp range. Moreover, ChIPmentation is less sensitive to the presence of large fragments because the DNA is fragmented during the tagmentation step.

Before starting the ChIP, the chromatin should be sheared to the desired size. Sonication should be optimized for each ChIP project since samples are different in their resistance to sonication. The following parameters should be considered during the optimization of chromatin preparation:

- Starting amount of cells
- Fixation
- Shearing optimization (followed by shearing assessment)

Starting amount of cells

Several variations of the protocol are available depending on the starting material.

- For chromatin preparation from 20,000 to 700,000 cells use the BATCH protocol. When possible, this option is preferred in order to limit tube-to-tube variability.
- For chromatin preparation from 10,000 to 20,000 cells use the INDIVIDUAL SAMPLES protocol. The shearing volume has to be adapted depending on the sonication tubes used.

Please check table 3 for more details.

Table 3. Description of the two protocols

STEP		BATCH PROTOCOL	INDIVIDUAL SAMPLES PROTOCOL	
Fixation	*Cell number/tube*	**20k - 700k**	**10k - 20k**	**10k - 20k**
	Final volume/tube	**1 ml**	**100 µl**	**100 µl**
	Tube	**1.5 ml**	**0.5/0.65 ml (Bioruptor)**	**0.2 ml (Bioruptor)**
Glycine + HBSS wash				
Lysis	*Cell number/tube*	**20k - 700k**	**10k - 20k**	**10k - 20k**
	Final volume/tube	**Multiples of (25 µl + 75 µl) HBSS***	**25 µl + 75 µl HBSS**	**25 µl + 15 µl HBSS**
	Tube	**0.5/0.65 or 1.5 ml (Bioruptor)**	**0.5/0.65 ml (Bioruptor)**	**0.2 ml (Bioruptor)**
Shearing	*Cell number/tube*	**10k - 300k**	**10k - 20k**	**10k - 20k**
	Final volume/tube	**100 - 300 µl**	**100 µl**	**50 µl**
	Tube	**0.5/0.65 or 1.5 ml (Bioruptor)**	**0.5/0.65 ml (Bioruptor)**	**0.2 ml (Bioruptor)**
	Bioruptor compatibility	**Pico or Plus**	**Pico or Plus**	**Pico**
Immunoprecipitation (not included)	*Cell number/tube*	10k - 100k	10k - 20k	10k - 20k
	Final volume/tube	200 µl	200 µl	200 µl
	Tube	1.5 ml	1.5 ml	0.2 ml (Bioruptor)
True MicroChIP kit	*Kit compatibility*	**Yes**	**Yes, preferred**	**Yes**
µChIPmentation kit		**Yes**	**Yes**	**Yes, preferred**

** According to the number of cells chosen for each IP, and the number of IPs*

Option A – BATCH PROTOCOL

The protocol describes the preparation of a batch of chromatin. The starting number of cells for one batch depends on the experimental plan (availability of cells and number of reactions to run). First, determine the number of IP (or shearing) reactions you will perform and the number of cells to be used per reaction. Then, start with fixation of a unique batch of chromatin accordingly:

Number of cells per chromatin batch = Number of cells per IP (from 10,000 to 100,000) x number of IPs.

Notes:

- *The minimum recommended number of cells to start the chromatin preparation (at fixation step) is 20,000 cells. This is enough for 2 immunoprecipitation/sonication samples, each of 10,000 cells.*
- *The maximum recommended number of cells to start the chromatin preparation (at fixation step) is 700,000 cells. If more cells are needed, then proceed with a separate chromatin preparation.*

Option B – INDIVIDUAL SAMPLES PROTOCOL

The µChIPmentation kit for histones and the True MicroChIP kits allow successful ChIP on as low as 10,000 cells per reaction, starting from the fixation step. The fixation step is performed in a smaller volume and directly in the shearing tube.

Note: *The maximum recommended number of cells to start the chromatin preparation (at fixation step) is 20,000 cells. If more cells are needed, then proceed with the batch protocol.*

The choice of the shearing tube will have an impact on this protocol. Depending on the size of the shearing tubes used, the shearing volume will need to be adapted as described in the protocol.

The different options are:

- 0.2 ml microtubes (Cat. No. C30010020) they are only available for Bioruptor Pico. They are recommended when using µChIPmentation, where they allow a single-tube approach for

efficient ChIP. In this protocol the number of sample transfer is limited: chromatin preparation, immunoprecipitation and tagmentation are performed in one single microtube, in order to reduce sample loss.

- 0.5ml microtubes (Cat. No. C30010013): they are recommended when using Bioruptor Plus. They are easier to handle but are not compatible with a thermocycler for tagmentation step. Therefore they are not suited for µChIPmentation single-tube approach, but can be used in combination with True MicroChIP kit.
- 0.65ml microtubes (Cat. No. C30010011): They are easier to handle and associated with Bioruptor Pico. But they are not compatible with a thermocycler for tagmentation step. Therefore they are not suited for µChIPmentation single-tube approach, but can be used in combination with True MicroChIP kit.

Fixation optimization

The described protocols use a mild fixation sufficient for histone proteins. Cells should be re-suspended in PBS prior to the fixation to avoid cell clusters formation upon fixation to ensure a proper shearing efficiency.

Formaldehyde is the most commonly used cross-linking reagent ideal for two molecules which interact directly. The fixation time can depend on your target of interest and might require an additional optimization (usually between 8 and 10 minutes for histones). Please note that a longer fixation may lead to chromatin resistant to sonication. Adapt sonication time accordingly.

If different fixation times will be tested, we recommend starting with a corresponding number of chromatin preparations and testing different sonication settings for each preparation.

Shearing optimization

The length of sonication time depends on many factors (cell type, cell density, sample volume, fixation time). Hence it is important to optimize the sonication conditions for each new ChIP project.

During the sonication, the mean size of DNA fragments will decline progressively approaching a lower limit of 100-150 bp (mean size of the smear). It is recommended to choose a sonication time before reaching this lower limit. **As best practice, choose the shortest sonication time resulting in a satisfactory shearing and ChIP efficiency (highest recovery/ lowest background)**. Avoid over-sonication, as it may lead to a drop of efficiency in ChIP experiments.

Note that the chromatin shearing from a limited number of cells is usually quite efficient and does not require a long sonication time. Ensure that only the recommended tubes are used for sonication. Please refer to the following guide:

https://www.diagenode.com/files/organigram/bioruptor-organigram-tubes.pdf

Caution: It is very important to carefully follow Diagenode's recommendations, because many parameters can influence the sonication efficiency, such as:

- The Bioruptor reference - sonication tubes recommended for the Bioruptor Pico are different from the tubes recommended for the Bioruptor Plus.
- The tube size - switching to another type of tubes (e.g. from 0.65 ml to 0.2 ml tubes) would require an additional optimization.
- The sample volume - the optimal volume is different for each tube reference. This sample volume should be kept consistent between experiments to ensure reproducible results.

Any change in one of those parameters can lead to inefficient shearing and lack of reproducibility.

Chromatin shearing assessment

The analysis of shearing efficiency is not obvious when working with 10,000 cells due to the low amount of DNA recovered.

We recommend using the Fragment Analyzer or Bioanalyzer (Agilent) for the size assessment due to a high sensitivity of these systems comparing to a conventional agarose gel electrophoresis.

We highly recommend using MicroChIP DiaPure columns (not included in the kit) for DNA purification. The eluted DNA is enough concentrated for accurate sizing from 1,000-2,000 cells equivalent (Figure 1). The minimum DNA concentration allowing an appropriate visualization of sheared DNA on Fragment Analyze and Bioanalyzer is within 2-10 ng/µl.

However, some inconsistencies between Fragment Analyze and Bioanalyzer might be observed. The Bioanalyzer traces are sometimes biased towards the high-molecular weight fragments (Figure 2).

The conventional agarose gel is not sensitive enough to visualize low amounts of DNA. If chromatin shearing assessment is performed using agarose gel electrophoresis, a minimum input of 40,000-50,000 cells is required for reliable assessment (Figure 1). Therefore, several replicates, equivalent to 40,000-50,000 cells, should be pooled before loading onto agarose gel. We recommend using a thin agarose gel for better signal visualization. Both the pre- and post-staining of the agarose gel with ethidium bromide or SybrSafe dye can be used for visualization of sheared fragments. Some slight differences might be observed between post- and pre-stained gels. Post-staining eliminates any possibility that the dye interferes with the migration and ensures an even background noise. However, pictures are usually less clear and bright with some background noise. If pre-stained agarose gels are used, it is advised that the electrophoresis buffer contains the stain in the same concentration as in the gel. If the stain is present in the gel but not in the buffer, the gel will result in uneven staining because the free dye migrates towards the top of the gel leaving the bottom part with no stain. Therefore, the background noise becomes non-uniform.

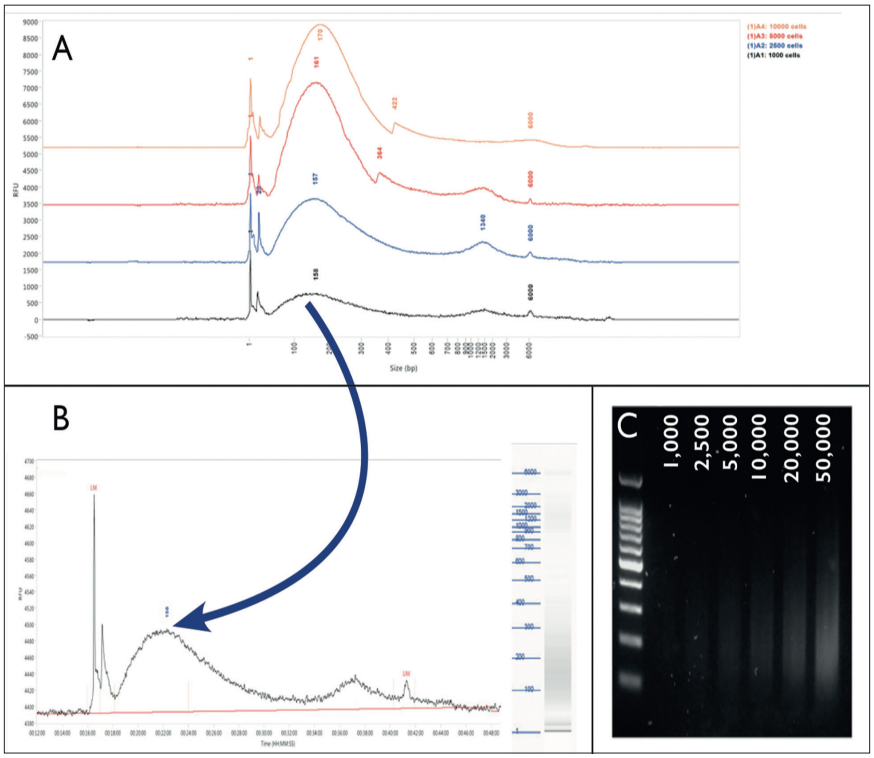


Figure 1. The sensitivity of chromatin shearing analysis using the Fragment Analyzer and agarose gel.

HeLa cells were fixed with formaldehyde and chromatin was prepared accordingly to Chromatin EasyShear - High SDS kit (Cat. No. C01020012) using the Bioruptor Pico for 8 cycles. After reversal of cross-linking and purification, samples equivalent to 1.000 up to 50.000 cells (as indicated) were separated using the Fragment Analyzer with the HS NGS Fragment Kit (A&B) or agarose gel electrophoresis (C).

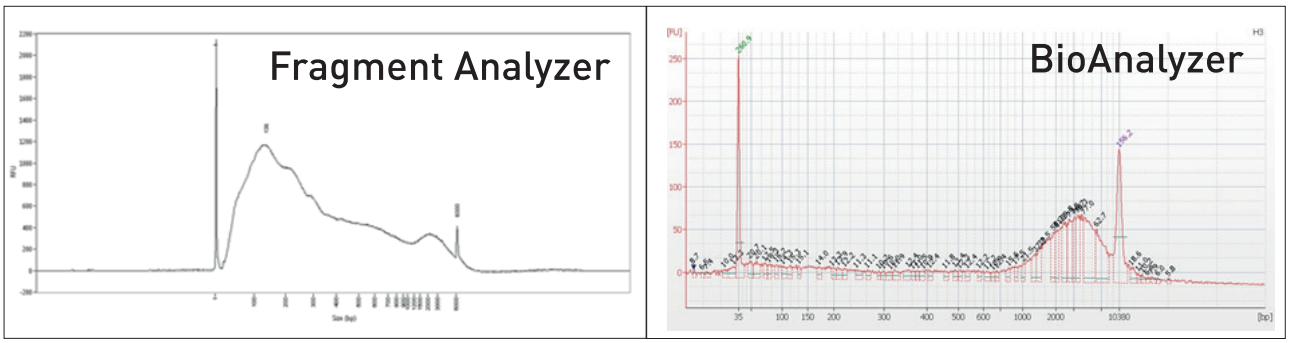


Figure 2. Inconsistencies between Fragment Analyzer and Bioanalyzer: the Bioanalyzer trace is biased towards the high-molecular- weight fragments.

HeLa cells were fixed with formaldehyde and chromatin was prepared accordingly to Chromatin EasyShear - High SDS kit (Cat. No. C01020012) using the Bioruptor Pico for 8 cycles. After reversal of cross-linking and purification, an equivalent of 2.000 cells was analyze either using the Fragment Analyzer or BioAnalyzer.

When using Fragment Analyzer or Bioanalyzer (Agilent), please follow the manufacturer's instructions. Please keep in mind that traces are log-based, so a large distribution of higher molecular weight fragments are compacted into a much smaller area of the trace as compared to the smaller size fragments leading to a visual misinterpretation of fragment distribution. If high molecular weight fragments are present, it is recommended estimating a molar ratio between fragments in a

desired range and higher molecular weight fraction. The molarity allows estimating a number of molecules in a particular range. The presence of high molecular weight fragments up to 15% (molar ratio) is acceptable for the majority of ChIP-seq projects (Figure 3).

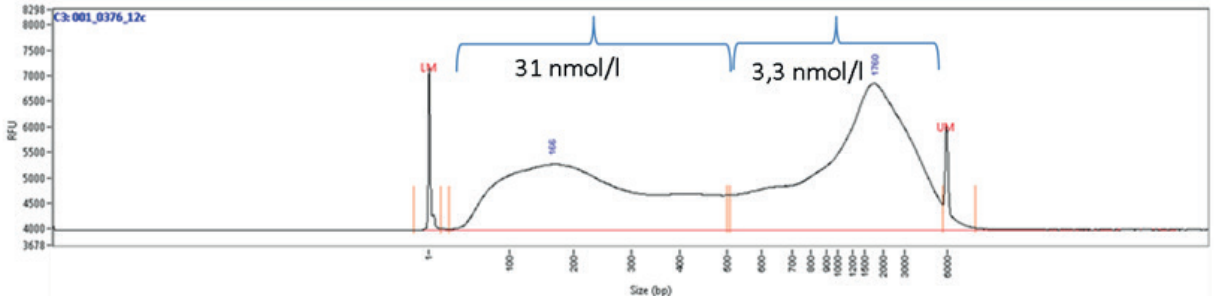
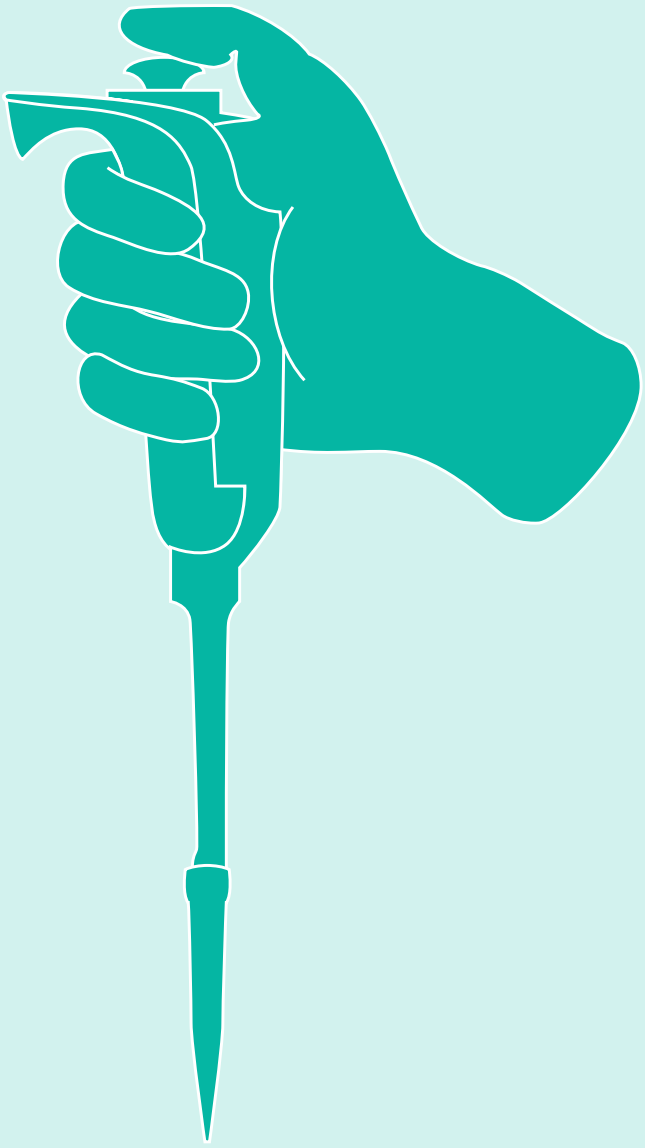


Figure 3. Estimation of a molar ratio between fragments in a desired range higher molecular weight fraction.

HeLa cells were fixed with formaldehyde for 10 min and chromatin was prepared according to Diagenode's protocol (Cat.No. C01020013). Samples were sonicated for 12 cycles of 30" ON/30" OFF with Bioruptor Pico using 1.5 ml Bioruptor microtubes with caps (Cat. No. C30010016) followed by de- crosslinking and DNA purification. The fragment size was assessed using Fragment Analyzer. The molar content of fragments in the range 100-500 bp and 500-5.000 bp was estimate d showing that large fragments do not exceed 15%.

For accurate size determination of the chromatin fragments, reverse crosslinking, including RNase treatment followed by DNA purification, is needed (Figure 4).

PROTOCOL



OPTION A - BATCH

Compatible with µChIPmentation kit for histones and with True MicroChIP kit

STEP 1

Cell collection and DNA-protein cross-linking from a batch of cultured cells

Day 1  *1 hour*

The protocol below describes the preparation of a batch of chromatin. It can be applied to a batch from 20,000 up to 700,000 cells, which can be further used for desired number of IPs, each IP being performed with the desired number of cells (from 10,000 to 100,000).

A1.1 Place PBS, cell culture medium and trypsin-EDTA (required for adherent cells) at 37°C. Ice-cold HBSS will be used at this step.

For adherent cells:

A1.2 Remove the cell culture medium and rinse the cells with pre-warmed PBS. Gently shake the flask for 2 minutes.

A1.3 Remove the PBS and add sterile **trypsin-EDTA** to the culture flask to detach adherent cells from the bottom. Add enough trypsin-EDTA to completely cover the cells. Gently shake the culture flask for 1-2 minutes or until the cells start to detach.

NOTE: *The time needed may depend on the cell type. Do not continue trypsin treatment longer than necessary as prolonged treatment may damage the cells. Regularly check if the cells start to detach.*

A1.4 Immediately add fresh culture medium to the cells when they are detached. For correct inactivation, the volume of medium must be 2x the volume of trypsin-EDTA. Transfer cell suspension to a 15 ml tube and go directly to point A1.5 of the protocol.

For suspension cells:

A1.5 Centrifuge for 5 minutes at 500 x g at room temperature and remove the supernatant.

A1.6 Resuspend the cells in **1 ml of cell culture medium** (RT) and count the cells. You should have between 20,000 and 700,000 cells per ml of cell culture medium.

NOTE: *At this step it is possible to transfer the resuspended cells in a clean 1.5 ml tube for an easier handling.*

A1.7 Under a fume hood, add **27 µl of 37% formaldehyde** per **1 ml** of sample. The final concentration of formaldehyde should be 1%. Invert tubes immediately 2-3 times to ensure complete mixing.

NOTE: *Always use fresh formaldehyde.*

A1.8 Incubate for 8 minutes at room temperature to allow fixation to take place.

A1.9 Add **115 µl of Glycine** to the sample. Mix by gentle inversion of the tube 4-5 times. Incubate for 5 minutes at room temperature to stop the fixation. Keep everything at 4°C or on ice from now on.

A1.10 Centrifuge samples at 300 x g for 10 minutes at 4°C.

NOTE: *We recommend the use of a swing-out rotor with soft settings for deceleration.*

A1.11 Aspirate slowly 1.1 ml of the supernatant to leave **30 µl** of solution. Do not disturb the pellet.

A1.12 Add **5 µl of protease inhibitor cocktail** to **1 ml of ice cold HBSS** and add it to the cell pellet. Invert tubes 4-5 times.

NOTE: *When working with 100,000 cells and more per batch, you should gently vortex to completely re-suspend the cells.*

A1.13 Centrifuge samples at 300 x g for 10 minutes at 4°C.

A1.14 Carefully discard the supernatant and keep the cell pellet on ice. Proceed directly to cell lysis.

NOTE: *We strongly recommend using freshly fixed cells for preparation of sheared chromatin prior to ChIP. If not possible, the fixed cells can be stored at -80°C for up to 2 months.*

OPTION A - BATCH

STEP 2

Cell lysis and chromatin shearing of a batch of cells

1 *Day 1* *30 min. to1 hour*

CAUTION: *Make sure that there are no crystals in the Lysis Buffer tL1 before using it. Gently warm at room temperature and mix until crystals disappear.*

A2.1 **25 µl of complete Lysis Buffer tL1** will be needed for **each IP** reaction. Determine accordingly the total volume of Lysis Buffer tL1 needed.

NOTE: *It is possible to use from 10,000 to 100,000 cells per IP, therefore the volume of Lysis Buffer tL1 to prepare is 25 µl for 10,000 to 100,000 cells, depending on the experimental plan.*

A2.2 Prepare **complete Lysis Buffer tL1** by adding the protease inhibitor cocktail 200x (e.g. add 1 µl of protease inhibitor cocktail 200x to 200 µl of Lysis Buffer tL1). Keep the buffer at room temperature until use. Discard what is not used within a day.

A2.3 Add **25 µl of complete Lysis Buffer tL1** per **IP** reaction to the cells. Agitate manually the bottom of the tube to resuspend the cells and allow bubbles to form.

A2.4 Incubate for 5 minutes on ice to ensure complete cell lysis.

A2.5 **75 µl of complete HBSS** will be needed for **each IP** reaction. Determine accordingly the total volume of complete HBSS and prepare it by adding the protease inhibitor cocktail 200x (e.g. add 3 µl of protease inhibitor cocktail 200x to 600 µl of HBSS). Add 75 µl of complete HBSS per each IP reaction. The volume of each individual sample for IP should be 100 µl (25 µl tL1 and 75 µl HBSS).

NOTE: *Ensure that there are no crystals precipitates in samples. Otherwise, gently warm sample to room temperature until crystals disappear.*

A2.6 Transfer the cell suspension to sonication microtubes and if needed split it into aliquots.

The following tubes can be used for sonication:

Microtubes	Cat. No.	Bioruptor model	Sample volume
0.65 ml	C30010011	Pico	100 µl
1.5 ml with caps	C30010016	Pico	100-300 µl
0.5 ml	C30010013	Plus	100 µl
1.5 ml TPX	C30010010-300	Plus	100-300 µl

A2.7 Shear the chromatin by sonication using the Bioruptor. Perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation. Choose the protocol which is adapted to your device:

- When using the **Bioruptor Pico**, an initial time-course experiment of **3-6-9** sonication cycles 30'' ON/30'' OFF is recommended.
- When using the **Bioruptor Plus**, an initial time-course experiment of **10-15-20** sonication cycles [30 seconds "ON", 30 seconds "OFF"] at High power is recommended.

A2.8 Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at 16,000 x g at 4°C for 10 minutes. Collect the supernatant which contains the sheared chromatin.

A2.9 Use the chromatin for STEP 3 - Chromatin shearing assessment or store it at -80°C for up to 4 months. Avoid freeze/thaw cycles.

OPTION B - INDIVIDUAL SAMPLES

Compatible with µChIPmentation kit for histones
and with True MicroChIP kit

STEP 1

Cell collection and DNA-protein cross- linking from low amounts of cultured cells

Day 1

1h30

The protocol below describes the chromatin preparation from individual samples from 10,000 up to 20,000 cells. The chromatin can then be used directly for one immunoprecipitation reaction.

For adherent cells:

B1.1 Pre-warm PBS, culture medium and trypsin-EDTA at 37°C.

B1.2 Remove the medium and rinse the cells with **pre-warmed PBS**. Gently shake the flask for 2 minutes.

B1.3 Remove the PBS and add sterile **trypsin-EDTA** to the culture flask to detach adherent cells from the bottom. Add enough trypsin-EDTA to completely cover the 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. Do not continue trypsin treatment longer

than necessary as prolonged treatment with trypsin may damage the cells. Regularly check if the cells start to detach.

B1.4 Immediately add fresh **culture medium** to the cells when they are detached. For correct inactivation, the volume of medium must be 2x the volume of trypsin-EDTA. Transfer cell suspension to a 1.5 ml tube.

For suspension cells:

Collect suspension cells in a 1.5 ml tube and go directly to point B1.5 of the protocol.

B1.5 Centrifuge for 5 minutes at 500 x g (at room temperature) and remove the supernatant.

B1.6 Resuspend the cells in **cell culture medium** and count them.

B1.7 Distribute cell suspension containing the desired cell number to microtubes of choice:

- 0.2 ml microtubes (Cat. No. C30010020) for single tube workflow associated with the Bioruptor Pico
- 0.65 ml microtubes (Cat. No. C30010011) associated with Bioruptor Pico
- 0.5 ml microtubes (Cat. No. C30010013) associated with Bioruptor Plus

Add cell culture medium to reach a final volume of 100 µl in each tube.

NOTES:
The choice of the shearing tube will have an impact on the rest of the protocol. See remarks before starting to choose the best option.

The number of cells per IP should be from 10,000 to 20,000 cells.

B1.8 Under a fume hood, add **2.7 µl of 37% formaldehyde** to each tube containing **100 µl** of cell suspension and mix gently.

B1.9 Incubate 8 minutes at room temperature with occasional manual agitation to allow fixation to take place.

NOTE: *The fixation time might require an additional optimization. Please refer to the "Remarks before starting".*

B1.10 Add **11.5 µl of Glycine** (white cap) to the cells to stop the fixation. Mix gently. Incubate for 5 minutes at room temperature to stop the fixation. Keep everything at 4°C or on ice from now on.

B1.11 Collect the cells by centrifugation at 300 x g for 10 minutes at 4°C.

NOTE: *We recommend the use of a swing-out rotor with soft settings for deceleration.*

B1.12 Aspirate slowly 85 µl of supernatant slowly to **leave 30 µl** of solution. Do not disturb the pellet. Take care to not remove these cross-linked cells.

B1.13 Prepare **complete HBSS Buffer** by adding the protease inhibitor cocktail 200x (e.g. add 7 µl of protease inhibitor cocktail 200x to 1.4 ml of HBSS). This complete HBSS Buffer will be used twice:

- 120 µl per tube at point **B1.14**
- And 15 µl per tube at point **B2.5** if using 0.2 ml microtubes, or 65 µl per tube at point **B2.5** if using 0.5/0.65 ml microtubes

B1.14 Wash the cross-linked cells with **120 µl of ice-cold complete HBSS** as follows:

- Add 120 µl of complete HBSS
- Gently mix to resuspend the cells
- Centrifuge at 300 x g for 10 minutes at 4°C (in a swing-out rotor with soft settings for deceleration). Discard 140 µl of supernatant in order to **leave 10 µl** of solution.

OPTION B - INDIVIDUAL SAMPLES

STEP 2

Cell lysis and chromatin shearing of individual samples

Day 1

30 min. to 1 hour

CAUTION: *Make sure that there are no crystals in the Lysis Buffer tL1 before using it. Gently warm at room temperature and mix until crystals disappear.*

B2.1 **25 µl of Lysis Buffer tL1** will be needed for **each tube**. Determine accordingly the total volume of Lysis Buffer tL1 needed.

B2.2 Prepare **complete Lysis Buffer tL1** by adding the protease inhibitor cocktail 200x (e.g. add 1 µl of protease inhibitor cocktail 200x to 200 µl of Lysis Buffer tL1). Keep the buffer at room temperature until use. Discard what is not used within a day.

B2.3 **Add 25 µl of complete Lysis Buffer tL1** to **each tube**. Agitate manually the bottom of the tube to resuspend the cells and allow bubbles to form.

B2.4 Incubate for 5 minutes on ice to ensure complete cell lysis.

B2.5 Add **complete HBSS** to the cell lysate, according to the type of shearing tube as described in the table below:

Microtubes	Cat. No.	Bioruptor model	Complete HBSS volume
0.2 ml	C30010020	Pico	15 µl
0.65 ml	C30010011	Pico	65 µl
0.5 ml	C30010013	Plus	65 µl

B2.7 Shear the chromatin by sonication. Perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation. Choose the protocol which is adapted to your device and tubes:

- When using the **Bioruptor Pico**, and 0.2 ml microtubes an initial time-course experiment of **3-6-9** sonication cycles 30'' ON/30'' OFF is recommended.
- When using the **Bioruptor Pico**, and 0.65 ml microtubes an initial time-course experiment of **3-6-9** sonication cycles 30'' ON/30'' OFF is recommended.
- When using the **Bioruptor Plus**, and 0.5 ml microtubes an initial time-course experiment of **10-15-20** sonication cycles [30 seconds "ON", 30 seconds "OFF"] at High power is recommended.

B2.8 Use the chromatin for STEP 3 - Chromatin shearing assessment or store it at -80°C for up to 4 months. Avoid freeze/thaw cycles.

STEP 3

Chromatin shearing assessment

Workflow for analysis of sheared chromatin:

- Reverse crosslinking
- RNAse treatment using RNAse cocktail
- DNA purification using MicroChIP DiaPure Columns (not provided, Diagenode, Cat. No. C03040001)
- Fragment size assessment

Reverse cross-linking (reagents included in the kit)

3.1 Take an aliquot, containing the required amount of sheared chromatin:

- If a microfluidic device (Fragment Analyzer or Bioanalyzer) will be used for size assessment, an equivalent of 1,000-2,000 cells (or higher) should be used
- If agarose gel electrophoresis will be used for size assessment, an equivalent of 40,000-50,000 cells, (or higher) should be used.If an individual sample does not contain enough cells, perform several reactions in parallel, and pool DNA obtained at the end of protocol before loading on agarose gel

3.2 Adjust, if needed, the volume of the sheared chromatin to **100 µl** using TE buffer

3.3 Add **100 µl of the Elution Buffer tE1** and **8 µl of Elution Buffer tE2** to each sample. Mix thoroughly and incubate samples at 65°C for 4 hours (or overnight) in a thermoshaker at 1300 rpm.

DNA purification using MicroChIP DiaPure Columns and RNase treatment

NOTE: *Before the first use of the DNA Wash Buffer, 24 ml of ethanol must be added to 6 ml of the Buffer. Never leave the bottle open during storage to avoid evaporation.*

3.4 Add **1 ml of ChIP DNA Binding Buffer** to each sample and mix briefly.

3.5 Transfer **0.6 ml** of the mixture to a provided spin column in a collection tube and centrifuge at ≥10,000 x g for 30 seconds. Discard the flow-through

3.6 Repeat step 3.5 again with the remaining 0.6 ml of the mixture.

3.7 Add **200 µl of DNA Wash Buffer** to the column. Centrifuge at ≥10,000 x g for 30 seconds.

3.8 Add **1 µl of RNase cocktail** directly to the center of the spin column membrane and incubate for 15 minutes at room temperature

3.9 Add **200 µl of DNA Wash Buffer** to the column. Centrifuge at ≥10,000 x g for 30 seconds.

3.10 Add **6 µl of DNA Elution Buffer** directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at ≥10,000 x g for 30 seconds to elute the DNA.

NOTE: *Up to 50 µl of DNA Elution Buffer can be used if less concentrated DNA is required for analysis.*

Fragment size assessment

3.11 Analyze the purified DNA using the preferred option:

- Fragment Analyzer
- Bioanalyzer
- agarose gel electrophoresis

Use high sensitivity kits accordingly to the manufacture's recommendation.

3.12 Chose the shortest sonication time resulting in a satisfactory shearing profile. Avoid over-sonication, as it may lead to a drop of efficiency in ChIP experiments.

FAQs

What is the composition of buffers included in the kit?

The composition of buffers is proprietary.

Is it possible to change the decross-linking protocol and use 5 minutes at 95°C to save time?

After heating at 95°C the DNA is single-stranded and therefore more difficult to analyze. It is not really compatible with agarose geland associated dyes, and Qubit or BioAnalyzer kits for ssDNA are not very accurate. Therefore in order to have shearing results that are trustable and easier to analyze you have to perform the decross-linking 4h at 65°C

Is RNase treatment mandatory for chromatin shearing assessment?

RNase treatment significantly reduces background caused by degraded RNA and improves visual assessment of shearing. The presence of degraded RNA in the sample might lead to mis-interpretation of the shearing. Smear below 100 bp is due to degraded RNA but not over-sheared DNA.

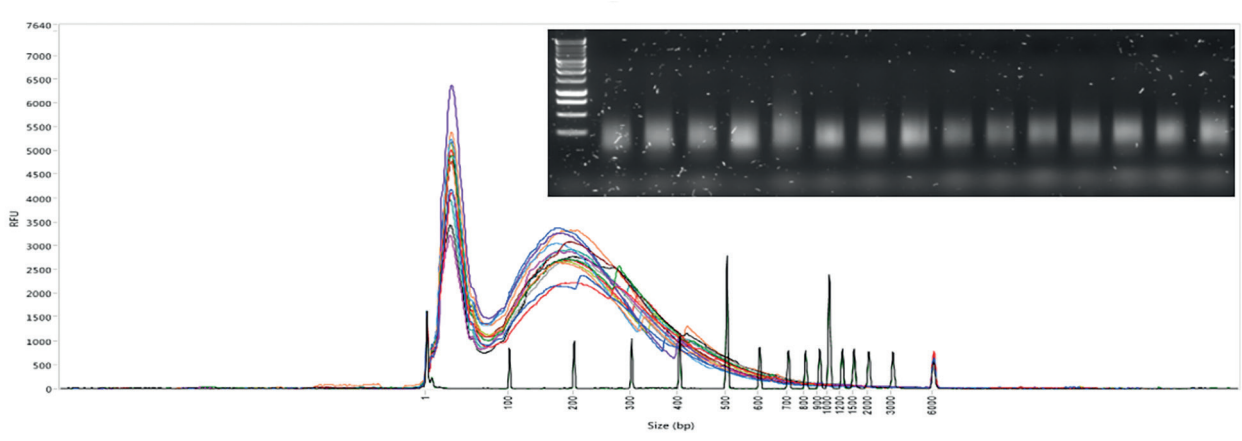


Figure 4. The presence of degraded RNA in the sample interfere with shearing assessment.

Chromatin from HeLa cells was prepared according to Diagenode's protocol. Samples were sonicated for 5 cycles of 30" ON/30" OFF Bioruptor Pico followed by de-crosslinking and DNA purification in the absence of RNase. The fragment size was assessed using agarose gel electrophoresis (100 bp ladder was loaded as the size standard) and Fragment Analyzer and High Sensitivity NGS Fragments kit (Agilent). A sharp peak below 100 bp correspond to degraded RNA.

Is reverse cross-linking mandatory for chromatin shearing assessment?

Size estimation of chromatin fragments without reverse cross-linking is not precise. The presence of the crosslinks retards electrophoretic migration resulting in a misinterpretation of fragment size (Figure 5). Therefore it is important to follow the decross-linking protocol as described in "Step 3 – chromatin shearing assessment".

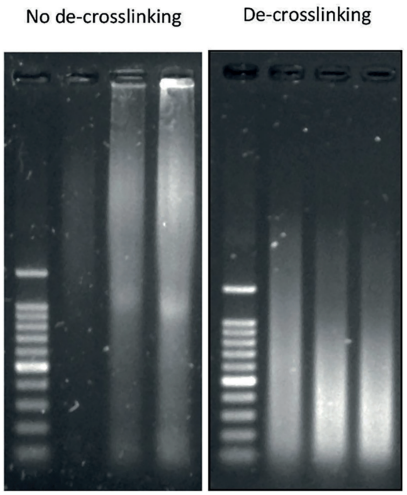


Figure 5. Reversing crosslinks is necessary for accurate size estimation.

HeLa cells were fixed with formaldehyde and chromatin was prepared accordingly to Diagenode's protocol. Samples were sonicated for 5, 10 and 15 cycles of 30" ON/30" OFF as indicated with the Bioruptor Pico using 1.5 ml Bioruptor microtubes with caps (Cat. No. C30010016). A 100 bp ladder was loaded as size standard. Left panel: non de-crosslinked chromatin. Right panel: de-crosslinked chromatin.

Related products

Product	Cat. No.
µChIPmentation kit for Histones	C01011011
True MicroChIP kit	C01010130
MicroChIP DiaPure columns	C03040001
Bioruptor Pico	B01060010
0.2 ml microtubes	C30010020
0.65 ml microtubes	C30010011
1.5 ml microtubes with caps	C30010016
Bioruptor Plus	B01020003
0.5 ml microtubes	C30010013
1.5 ml TPX microtubes	C30010010-300

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