Instruction Manual Note Vs2 (22.04.2008)

Diagenode sa
CHU, Tour GIGA, 3e étage
Avenue de l'hôpital, 1
4000 Liège - BELGIUM
Tel. +32 (0)4 364 20 50
info@diagenode.com
www.diagenode.com

Bioruptor ™

User manual

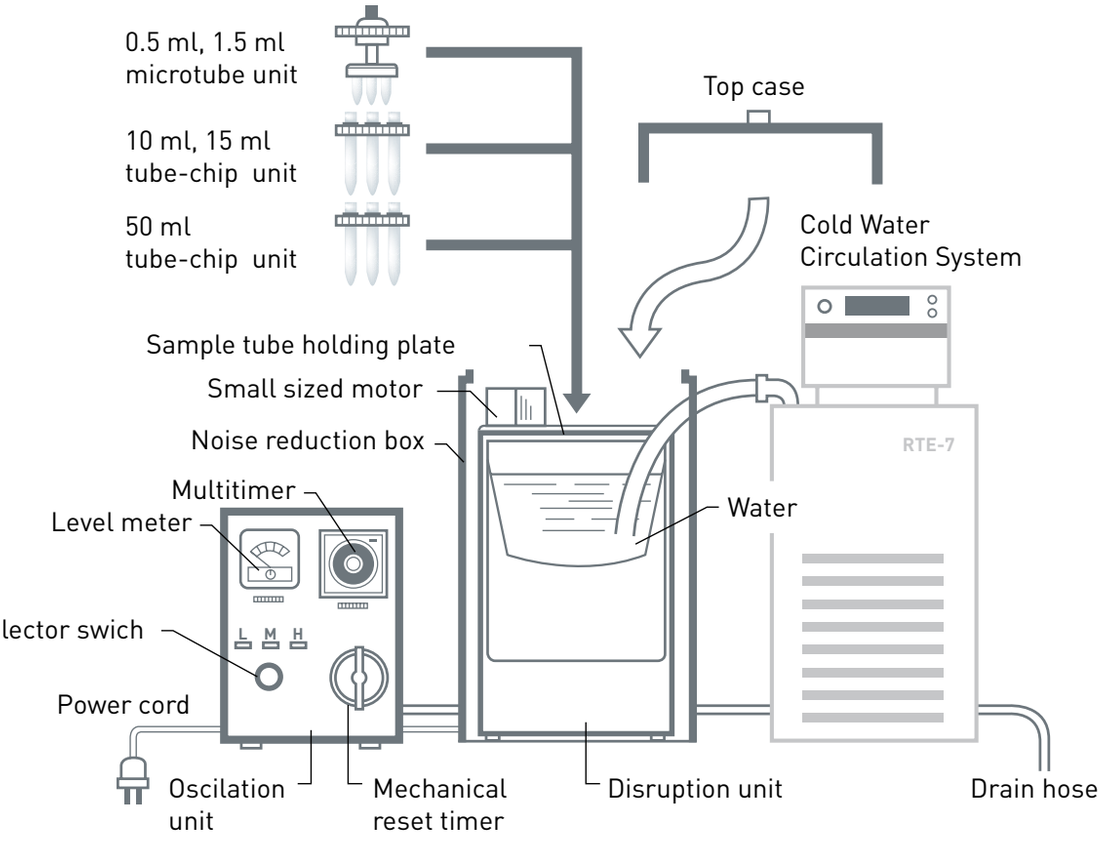
diagenode

Content

MANUAL

The complete Bioruptor system

with the different adapter units and the optional cold water circulation system.



Introduction

Advantages of the bioruptor - over the other sonicator apparatus:

- With the bioruptor the quality of the sheared chromatin is greater: a compact pack of chromatin fragments of the right size is obtained rather than a smear. In the chromatin sheared using the bioruptor, the amount of different DNA fragment sizes is reduced and the presence of too long DNA fragments is eliminated, these being a source of background in the subsequent ChIP experiment. Thus, the high quality of the sheared chromatin obtained with the bioruptor is essential to get better results in ChIP, by decreasing dramatically the background noise. The way to test the sonication efficiency is to look at the extent of DNA fragmentation using an agarose gel.
- With DNA sheared with the bioruptor, the reproducibility is close to 100%.
- Note that the bioruptor can also be used for many other applications.

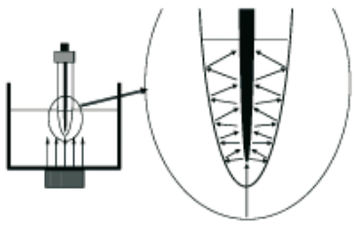
What is the difference between the Bioruptor and a traditional probe system?

The traditional sonicators imply a probe directly in contact with the biological sample. This has major drawbacks in terms of reproducibility as the energy of sonication depends on the depth of the sonication probe in the liquid. Moreover the probe system is tedious to work with (production of foam) and only one sample can be treated at the time. Contamination between different samples is frequent. Additionally, the probe system is generating aerosols which are not tolerated by biosafety rules.

The **Bioruptor** System is based on a water bath with high power ultrasound generating elements located below the tank.
With the Bioruptor 3 to 12 closed tubes can be sonicated together and the continuous rotation of tubes allows even distribution of the energy.
With a better control of the parameters, the **Bioruptor** enables the automation of the sonication step which guarantees higher reproducibility and constant results.
The frequency of the ultrasounds produced by the **Bioruptor** and a probe sonicator is equivalent (20 kHz).

What is the effect of ultrasound on biological samples?

A generally accepted view is that ultrasounds are producing a gaseous cavitation in the liquid. This term describes the formation of small bubbles from dissolved gases or vapors due to alteration of pressure. These bubbles are capable of resonance vibration and produce vigorous eddying or microstreaming which is sufficient to break cells. Also, the fragmentation of DNA takes place as a consequence of mechanical stress or shear from the bubbles.
With a sonicator probe, the micro-streaming phenomenon is limited to the vicinity of the probe, whereas for the **Bioruptor**, the whole volume of water present in the tank is exposed to ultrasounds.
For tubes of 15 ml or 50 ml, the transfer of the ultrasounds inside the tubes is facilitated by a metallic bar in contact with the sample. This metallic bar is not a probe (no corrosion problems) but "reflects" the ultrasounds originated from the water bath and improves the sample sonication efficiency by a patented resonance system. The figure below schematizes the resonance of the ultrasound on the metallic bar.
(To better understand the sonication process, see useful references (1, 2)).



THE BIORUPTOR

Read entirely this user manual to get familiar with all the components of the Bioruptor.

Water bath:

The bath is the critical part of the machine and you must take particular care of it. The bath must stay stand-up, especially when you move it. **If you tilted or moved it too harshly, some glued component could detach and the sonication efficiency would be quickly falling down.**
You might have to transport the apparatus from a place to another for any reason. It is imperative to keep the tank upside right during the transport at any time.

Water level in the tank

The transfer of the ultrasounds produced by the generators located below the tank to the samples is done through a water bath.

- The level of the water has been optimized and should always reach the blue line (sticker on the wall of the tank)
- Tap water or distilled water can be used to fill the tank.

Maximum water level

Temperature of the water

Propagation of ultrasounds in a liquid unavoidably produces heat. Therefore, to ensure best preservation of the samples, it is necessary to start the sonication process with cold water in the water bath. The maintain of temperature during the sonication is obtained either by manual or automatic temperature control.

Manual temperature control

A "pre-cooling" of the Bioruptor's tank with crushed ice 15 minutes before starting the first round of sonication is advised to avoid too quick water heating due to thermal inertia (the tank and the ultrasound generating elements are generally stored at room temperature).
Fill now the water bath up to the indicated level with cold water We suggest you to keep a stock of water at 4°C (fridge or cold room). The crushed ice floating in the water should not exceed 0,5cm and the total water level (water and ice).
At the end of a typical sonication time (10 minutes, cycles of 30 seconds "ON" & 30 seconds "OFF"), the temperature in the water bath should stay below 10°C.
Note:

The permanent installation of the Bioruptor in a cold room is possible, although not sufficient to avoid the temperature increase due to sonication. This location would only suppress the "pre-cooling" step described above.

Automatic temperature control
A refrigerated circulation bath can be used to guarantee the automatic temperature control of the water bath during the whole sonication process.
The optional circulation bath RTE-7 features two pumps ("IN" and "OUT") and produces a regular water flow with a constant water level in the tank.

An additional regulating valve (Connectors kit for Thermo RTE-7D1) is adapted on the water circuit going from the refrigerating unit to the Bioruptor. In this way, the water flow can be reduced to an optimal level.
Keep the water flow tiny to not interfere with the resonance process in the water bath (Flow around 500 ml/minute).
This instrument can be ordered directly through Diagenode with all the required tubing (See price list).

Motorised lid:

The lid ensures the optimal position of the different tubes in the water bath during sonication.
The blue row is always easily placed into its location (see picture).
The motor (white arrow, see picture) keeps all the samples in constant rotation.
Avoid the immersion of the motor into the water.
When in motion, do not hamper the rotation of the blue row.

Tube Holders:

Several sizes of tubes can be used with the Bioruptor.
The maximum and minimum sample volume to be used with each container is given in the table below.

The 0.5 ml and 1.5 ml tubes are simply closed and installed in the rotor.
For the sonication of larger volumes (10 ml, 15 ml, 50 ml tubes), a **stopper with a metallic bar has to be used** for a better resonance efficiency. The metallic bar does not vibrate but "reflects" the ultrasounds originated from the bottom of the tank and initiates a resonance process (see introduction).

	Maximum volume per tube (or cup)	Minimum volume per tube (or cup)
0.5 ml tube	100 µl	10 µl
1.5 ml tube	300 µl	100 µl
15 ml tubes	2 ml	500 µl
50 ml tubes (Falcon or Corning)	20 ml	3 ml
50 ml tubes (Nalgene)	8 ml	1 ml

Controller:

The controller is the main unit and allows the user to direct the water bath. The specifications of the apparatus are written at the end of the manual.

Transformer:

The machine rating voltage is 100Volt. If the National Grid is 220Volt in your area, you have to lay a 220 Volt – 100 Volt transformer between the machine and the power socket.
The machine is provided with the good transformer integrated into an IEC power cord.
This transformer must be always used if you are in a 220 V area, otherwise you would destroy the machine.

0.5 ml microtube unit (MAT-05)

Turn the lower part of the microtube holder of counter clockwise, and take it off.
Place microtubes in the unit and attach again the lower part to the upper one.
To guarantee homogeneity of Chromatin Shearing the holder should always be completely filled.
Thin-walled 0.5 ml tube specially adapted for thermal cyclers could be used for sonication but all new material has to be tested with water-filled tubes in a typical sonication. Once selected, stick to a particular brand of tube for reproducibility.

1.5 ml microtube unit (MAT-15)

See above. To ensure reproducibility, always use the same brand of Eppendorf tubes.
The 2 ml Eppendorf tubes should not be used with the Bioruptor.
All Eppendorf tubes are generally in polypropylene. Special Eppendorf tubes in TPX plastic with a slightly better ultrasound transfer rate are available from Diagenode (See price list).
To guarantee homogeneity of Chromatin Shearing the holder should always be completely filled.

10 ml and 15 ml tube units

For 10 ml and 15 ml tubes, the sample holding plate is the NG-6. This gear plate can accommodate up to 6 tubes (UCD-200) and up to 12 tubes (XL-2006).

The holding plate should always be completely filled.
The complete resonance chip (including O ring) can be sterilized in autoclave. After more than 20 autoclave sterilization the O ring might need to be replaced (Spare part P-10, see price list).

10 ml tubes

O ring for the 10 ml chip unit

15 ml tubes

The adaptors for 15 ml tubes are available in two dedicated models: blue (Falcon tubes) and orange (Corning tubes). If you use another brand of tube, please try the one which fits the best (the metallic bar should not touch the wall of the tube).

Removing the black knob, it is possible to replace the O-ring.

Notes:
- The **15 ml tube** (Falcon or Corning): "Hard" plastic (Polystyrene or polyethylene-Crystal clear tube) can be used as well as "soft" plastic (Polypropylene, more white aspect), but you should stick to one kind as transfer of ultrasonic waves is different (Hard plastic is more efficient).
- Special 15ml tubes in TPX plastic with a better ultrasound transfer rate are available from Diagenode (see price list).
- When using the 15 ml tubes, do not forget to insert the **aluminium ring** to ensure an optimal position of the tube during sonication.

50 ml tube units

Three different models of adaptors for 50 ml tubes are available.
For these tubes, the sample holding plate is the NG-50-3 This gear plate can accommodate up to 3 tubes.

Nalgene type
The Nalgene tube in polycarbonate has a conical shape which is well adapted to the use in the Bioruptor. These tubes, although more expensive than the classical 50 ml tube (Falcon or Corning), offer the best efficiency in term of transfer of the ultrasound energy. These tubes can be ordered directly from Diagenode (Ref: 3015-0050).

Removing the black knob, it is possible to replace the O-ring (Ref P-22A).

Falcon or Corning Type: FT-50WS or CT-50WS

The adaptors for 50 ml tubes are available in two dedicated models: blue (Falcon tubes) and orange (Corning tubes). If you use another brand of tubes, please try the one which fits the best (The metallic bar should not touch the wall of the tube).

Removing the black knob, it is possible to replace the O-ring (Ref P-22B).

Note:
The quality of the 50 ml Corning tube: "hard" plastic (polyethylene-ref Corning 430304) can be used as well as "soft" plastic (Polypropylene, ref. Corning 430290) but you should stick to one kind as transfer of ultrasonic waves is different (hard plastic is more efficient).
For Falcon tubes, only polypropylene tubes are available.

Control Unit

The control unit allows the automatic production of "ON" & "OFF" cycles to preserve the samples from rapid heating due to ultrasounds. This can be done by the Multi-timer located on the upper right part of the control unit.
The setting of the "OFF" time is done by the green needle which can be handled by turning the external surface of the dial.
The setting of the "ON" time is done by the red needle which can be handled by turning the internal knob of the dial.
The unit (min 0 sec) can be modified by using a screw driver (see white arrows).

The Bioruptor is active as soon as you have turned the mechanical timer of the control unit clockwise and set the time (Maximum time: 15 minutes.).
The time set is the total time which is the sum of the "ON" and "OFF" cycles.
Alternatively, turning the timer knob counter clockwise will set the Bioruptor on a permanent position (always cycles "ON" and "OFF").
To stop the process, manually set the timer knob on vertical position.

Output selector switch:
There are three power settings for the sonication process.
These can be automatically switched by the power knob:

L:	Low	130 W
M:	Medium	160 W
H:	High	200 W

A level meter is also present on the control unit to visualize the sonication intensity.

OPTIMIZATION OF SONICATION PARAMETERS

The Bioruptor being a research instrument, it allows to select the different parameters of sonication. Once found for a particular biological sample (fixed sample volume and density), the Bioruptor will ensure a excellent reproducibility of the whole process.
The determination of the optimized parameters is done empirically by doing preliminary tests with samples of different density in the different tubes and by analyzing the results.

Critical points

The starting points are the sonication parameters which should give the expected results for a maximum of applications.

Water level in the tank: Precisely to the blue line

Temperature: Melting ice temperature (see above for details)

Power: For Chromatin Shearing set the Output selector switch on High (H)

Cycling parameters: Set the Multitimer on 30 seconds "ON" & 30 seconds "OFF"
(Red and green needles set on 0.5 Min.)

Tube type	Sample volume:
0.5 ml	100 µl
1 ml	300 µl
15 m (Polypropylene)	2 ml
50 ml (Nalgene)	8 ml
50 ml tubes (Falcon or Corning)	20 ml

Optimization test

Obtaining reproducible sonication results with biological material implies a strict quality control of the sonicated samples.
Biological materials as cell lysis extract, DNA solution or liposome suspension are a very complex mixture of molecules and the ability to disrupt by sonication one of the molecules in the mixture depends on the individual molecule concentration itself but also on the eventual presence of contaminants.
The viscosity of the solution is another factor that can affect the sonication efficiency, as the cavitation process (see introduction) is dependent of the viscosity coefficient of the solution.
Additionally, for ChIP assay users, the fixation step (percentage of formaldehyde, duration and temperature) is a key factor that can affect the chromatin shearing efficiency.

The best way to start optimization tests with the Bioruptor is to collect a large quantity of the biological material to be sonicated. This material should be enough characterized to be produced easily and available in a sufficient quantity to eventually repeat the optimization tests. For example, a nuclear extract should be always produced with the same protocol and generated with a fixed number of cells.

As the sample concentration is a key factor to calibrate the sonication efficiency, several aliquot of the starting material will be produced by dilution.

For example 6 aliquots of the starting material could be designed as follow: 1X, 5X, 10X, 20X, 50X, 100X.

The aliquot volume in each tube should be fixed and should correspond to the tube chosen for sonication (see table above in the starting points).

Sonicate the 6 tubes together using the sonication parameters listed on the starting points above and analyze the results.

If results show not enough disruption, either dilute again the samples stepwise or increment the total time of sonication.

If too disrupted, either concentrate sample or reduce the total time of sonication.

A fine tuning of the "ON" & "OFF" cycles and of the other sonication parameters can be done once a satisfactory result has been obtained on a specific sample concentration.

The optimal conditions should give excellent reproducibility in term of sonication results (i.e. fragment size) and preservation of the sonicated material (no overheating).

Important note when testing different sonication vessels:
15 and 50 ml tubes are sonicated in the Bioruptor with a metallic bar which triggers the resonance effect and improves the sonication efficiency (see introduction). In other words, if you optimized your sonication parameters in 15 ml tubes, the same sample concentration won't necessary be adapted to the sonication in Eppendorf tubes and higher sample dilutions for the Eppendorf tubes should be tested.

SONICATION PROTOCOL FOR MOLECULAR BIOLOGY

BIORUPTOR-Chromatin shearing for ChIP Assay using Drosophila embryos or cell lines

Protocol to obtain DNA fragments up to 500 bp long

1/ Starting material:

- For use in Falcon tubes, wet weight of biological material (Cell or nuclei pellets, embryos etc.) should not be more than 1 g per 10 ml buffer. Higher concentrations will lead to partially fragmented DNA.
- For use in Eppendorf tubes, customers have reported best results with a max. concentration of one million cells (HeLa, U20S or 293 cells) per 300 µl sonication buffer.

2/ Sonication parameters:

a. Bioruptor always on "High" (H).

b.1. Sonication in 1.5 ml Eppendorf tubes filled with maximum 300 µl sonication mixture.
Fill the tank with cold water (4°C), supplement with 0.5 cm crushed ice.
Sonicate for 6 to 8 minutes total time (30 seconds "ON", 30 seconds "OFF").

b.2. Sonication in 15 ml Falcon tubes filled with maximum 2 ml sonication mixture.
Fill the tank with cold water (4°C), supplement with 0.5 cm crushed ice.
Sonicate for 10 to 12 minutes total time (30 seconds "ON", 30 seconds "OFF").

c. Reverse cross-links and analyse on gel.

3/ Notes:

- If a high molecular weight DNA smear is observed instead of a high proportion of 500 bp DNA fragments, the amount of starting material (Cells or Embryos) is too high.
- This protocol is a starting point and the total time of sonication and/or "ON"-"OFF" cycling parameters should be modified. For example, yeast cells might require longer sonication times.

Protocol to obtain 200-350 bp DNA fragments

1/ Starting material:

- Starting material should be fixed a bit longer than usual to protect proteins from high energy ultrasonic disruption. This could contribute to the preservation of epitopes for the subsequent immuno-precipitation step.
- Sonicate in 15 ml Falcon tubes filled with 2 ml sonication mixture.
- Wet weight of biological material (Cell or nuclei pellets, embryos etc.) should not be more than 1 g per 10 ml buffer. Higher concentrations will lead to partially fragmented DNA.

2/ Sonication paramaters:

a. Bioruptor on "High" (H).
b. Fill the tank with cold water (4°C), supplement with 0.5 cm crushed ice.
c. Sonicate for 15 minutes total time (30 seconds "ON" & 30 seconds "OFF").
d. Remove the water in the tank with a cup.
e. Fill again with cold water and crushed ice as above.
f. Sonicate again for 15 minutes total time (30 seconds "ON" & 30 seconds "OFF").
g. Reverse cross-links and analyse on gel.

Small DNA fragments should be obtained (200-350 bp).

3/ Notes:

If a high molecular weight DNA smear is observed instead of small 350-200 bp DNA fragments, the amount of starting material (Cell pellet or embryos) is too high.

BIORUPTOR-Chromatin shearing for ChIP Assay using yeast

First Protocol

1/ Starting material:

A 50 ml yeast culture (0.6 to 0.8 OD) is pelleted after the fixation step which was carried out in 1% formaldehyde for 10 minutes at room temperature.
The pellet is beaded (mechanical shearing) following standard procedures to remove cell walls.

The fraction containing the chromatin (2 ml in sonication buffer containing SDS or not, depending on the application) is transferred to a 15ml tube (Corning or Falcon) and closed with the stopper (metallic bar). The bar tip should not touch the tube walls.

2/ Sonication parameters:

a. **Bioruptor tank:** pre-cooled and filled with cold water supplemented with 0.5 cm floating crushed ice (not more than the blue line, See "Bioruptor Quick Overview").
b. **Bioruptor power setting**: on position "high": 30 seconds "ON" (red needle) & 60 seconds "OFF" (green needle).
c. **Sonication total time**: 10 minutes and 30 seconds (= 7 cycles, = 3 minutes and 30 seconds cumulative sonication time). Temperature of the water bath at the end of sonication procedure is around 10°C.

3/ Notes:

- For more precision, the general timer is switched off manually after 10.5 minutes (General timer on the permanent position, on the left).

- The DNA is then analysed on agarose gel after reversing the cross-link.
  DNA fragment size is 250 bp on average, reproducibility between tubes is excellent.

- A lot of attention has to be put on cell density which is crucial to achieve a good reproducibility.

- If DNA is not at the right size (or smeared), repeat the experiment either in increasing the total time or in reducing the cell density.

Second Protocol

1/ Starting material:

In this protocol, a lot of attention has been put on removing of the cell wall fragments as remaining cell walls in the sonication buffer appears to hamper the fragmentation of DNA by sonication. Furthermore, the sonication step is done at lower power (Medium switch on the Bioruptor).

The protocol of sonication has been optimized to minimize the damage to DNA-associated proteins for the subsequent immunoprecipitation.

- Yeast cell wall is first digested by Yeast lytic enzyme (YLE from ICN).
- The remaining spheroplasts are fixed (1% formaldehyde for 30 minutes to one hour, stop fixation with glycin), spun down and then beaded (glass beads) following standard procedures (buffer: 1% SDS, 10 mM EDTA, 50 mM Tris HCl (pH 8.0), 0.5 mM PMSF-EtOH, 0.8 g/ml pepstatin A, 0.6 g/ml leupeptin).
- After beading, the spheroplast extract is spun down and the supernatant (avoid to disturb the cell debris pellet) is transferred in an Eppendorf tube for sonication with the Bioruptor (300 µl per microtube, average cell concentration of 2x 10e7 per ml).

2/ Sonication parameters:

a. **Bioruptor tank**: pre-cooled and filled with cold water supplemented with 0.5 cm floating crushed ice (not more than the blue line, See "Bioruptor Quick Overview").

b. **Bioruptor power setting**: on position "**Medium**": 10 seconds "ON" (red needle) & 20 seconds "OFF" (green needle).

c. Sonication total time: 16 minutes and 30 seconds (= 25 cycles, = 4 minutes and 10 seconds cumulative sonication time).

3/ Notes:

- For more precision, the general timer is switched off manually after 16min 30sec (General timer on the permanent position, on the left).
- The DNA is then electrophoresed on agarose gel after reversing the cross-link.
  DNA fragments size is 350 bp on average, reproducibility between tubes is excellent.

TROUBLESHOOTING GUIDE

BIORUPTOR-CHROMATIN SHEARING (1)

Major critical steps	Questions	Answers & Comments	
Fixation step	What is the formaldehyde final concentration to use?	**1%-1.5%.**	**Make sure you perform the fixation step with the correct** formaldehyde concentration.
	How long is the fixation step?	**Fix for 15 minutes.**	**It is possible to fix for as little as 5 minutes and up to 30 minutes maximum (depending on your** protein of interest **for subsequent ChIP assay).**
	What is the temperature to use for fixation?	**Fix at RT.**	**Fixation can be performed at 4°C, RT, and 37°C.** **Make sure you perform the fixation step at the right temperature.**
	Are the washes after fixation important?	**Wash the fixed cells properly. Make sure you get rid of ALL the formaldehyde.**	**Alternatively: use glycine to stop the fixation.**
Cell lysis	Is cell disruption complete?	**Do not use too many cells in the cell lysis buffer.**	**Lyse about 5x 10e6 cells/1ml.**
Number of cells/shearing buffer volume	What is the amount of cells per shearing trial to use?	**1x $10e^6$ - 10x $10e^6$cells/ 300 µl** **3x 10^6 - 30x 10^6 cells/ 30 µl**	**Do not use a too high cell concentration.**
Shearing buffer	What is the key buffer component?	**Include detergent in the buffer.**	**Quality and quantity of detergent is important.**
Shearing step	How long is the shearing?	**Shear for 15 minutes.**	**It is possible to shear for as little as 5 minutes and up to 30 minutes maximum (for longer fixation period: you need a longer shearing).**
	What is the best cycle?	**30 seconds «ON» + 30 seconds «OFF».**	
	What is the best temperature for shearing?	**4°C.**	**Keep it cool all the time.**
	What is the best volume/tube for shearing?	**1.5 ml per 15 ml tube** **200 µl per 1.5 ml tube**	**Do not use a too big sample volume.**

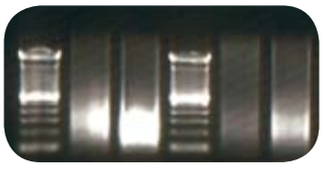
MANUAL

BIORUPTOR-CHROMATIN SHEARING (2)

Another critical steps	Questions	Answers &	Comments
Get a HIGH QUALITY picture of the shearing.	- Gel type. - Size accuracy.	**Test directly on agarose gel the DNA disruption (10µl/lane on 1% agarose gel).**	**For accurate size determination of the DNA fragments, the** reversion **of the cross-linking and DNA** precipitation **after phenol/ chloroform extraction is advised.**
	- Smears.	**Gel electrophoresis of cross-linked samples often gives smears on gel.**	**To obtain clearer image with accurate fragment size, reversion of the cross-linking is advised.**
	- Gel loading. - RNase treatment.	**The migration of large quantities of DNA on agarose gel can lead to poor quality pictures which do not reflect the real DNA fragmentation.**	**Do not load too much on a gel. Do not load more than 5µg/lane.** **Also treat the sample with RNase.**
	- Agarose gels.	**Agarose concentration.**	**Do not use more than 1% agarose gel and run slowly.**
		Running buffer concentration.	**1X TAE or TBE is preferred to 0.5X TAE which can lead to smears on gel.**
		Use a freshly prepared gel and fresh buffer.	**Do not re-use an old gel.**
	- Picture. - Exposure.	**The way the picture is taken really matters.**	**Make several pictures of the same gel. Choose the best.**

BIORUPTOR-CHROMATIN SHEARING (3)

1 2 3 4 5 6



top

bottom

Bioruptor™-chromatin shearing

Analysis on an agarose gel of the sheared chromatin obtained with the BioruptorTM from Diagenode.

U2OS cells are fixed with 1% formaldehyde (10 minutes at RT). One million cells are resuspended per 30 µl of lysis buffer prior to chromatin shearing. Each sample consists on 5x 10e6 cells per 1.5 ml tube. Samples are sonicated for 15 cycles of: [30 seconds "ON" / 30 seconds "OFF"] with the BioruptorTM from Diagenode (cat # UCD-200). Samples 1 and 2 are duplicates. The sheared chromatin is submitted to a cross-linking reversion, DNA purification and RNAse treatment. Then the samples are analysed on a 1% agarose gel. Two amounts of sheared chromatin are analysed (1.5 µl and 3 µl). The 100 bp DNA molecular weight marker is on lanes 1 and 4.

Note: Two pictures have been taken from the same samples analysed on the same gel: Top picture: samples 1 to 6. Bottom picture: samples 1 to 6. The quality of the picture is therefore dependent on the way the picture is taken (top versus bottom) and on the amount of sample analysed per lane (lanes 2 and 5: 1,5 µl versus lanes 3 and 6: 3 µl).

Conditions must be optimized for each cell type and fixation protocol.

Bioruptor UCD-200: Quick Overview

Critical steps	Solutions	Comments
Water level in the tank	Water level up to the blue line	- Do not add too much water or too little. - Never start the sonication without water in the tank.
Water temperature in the tank	Keep cool	- Add ice cold water to the tank and a small amount of crushed ice (0.5 cm maximum). - Additionally, "pre-cool" the tank with crushed ice 15 minutes before starting the first round of sonication in order to reduce the water heating due to thermal inertia (if the tank is stored at room temperature).
Maximum sample volume per tube for each sonication trial	100 µl of sample	In 0.5 ml tubes (PCR tubes).
	300 µl of sample	In 1.5 ml tubes (Eppendorf tubes).
	2 ml of sample	In 15 ml tubes.
	8 ml of sample	In 50 ml Nalgene tubes.
Quality of the 15 ml tube	Falcon or Corning	Polystyrene or polyethylene (cristal clear tube in "hard" plastic) is much more efficient for the transfer of ultrasonic waves (see also TPX tubes p.8 and p.9).
Sonicating in 15 ml tubes	Do not forget to **insert the aluminum ring**	The ring is essential to ensure an optimal position of the tube during sonication.
Sonication process	Sonicate by cycles	Cycles of: [30 seconds "ON"& 30 seconds "OFF"] are advised for chromatin shearing.
Optimisation of sonication cycling time	Cycles dependent on the sample volumes and type of tubes	Cycles depend on the application (chromatin shearing, DNA shearing, RNA shearing, obtention of bacteria lysates or tissue lysates...).

1. Elsner, H. and Lindblad E. (1989) DNA, 8: 697-701.
2. Hughes D. and Nyborg W. (1962) Science, 138: 108-14.
3. S. Berger's Lab (Wistar Institute, Philadelphia)
4. J. Mellor's Lab (University of Oxford, UK)

ORDERING INFORMATION

Diagenode s.a. Europe, Asia & Australia
CHU, Tour GIGA B34, 3rd Floor
Avenue de l'Hôpital, n°1
4000 Sart-Tilman, Liège
BELGIUM
Phone: +32 (0) 4 364 20 50
Fax: +32 (0) 4 364 20 51
Email: info@diagenode.com

Diagenode Inc. USA
376 Lafayette Road
Suite 202
Sparta, NJ 07871
USA
Phone : +1 973 300 0976
Fax: +1 973 300 1862
Email: infousa@diagenode.com

Diagenode website: http://www.diagenode.com

Technical specifications:

Power Supply	100/115V AC 50-60 Hz
Power Consumed	MAXIMUM 450 Watt
Protective Devise	Fuses
Dimension (H x W x D)	40 x 50 x 40 cm
Capacity	6 tubes of 1.5 ml
Weight	18 kg
Timer	Mechanical Timer (up to 15 min)
Accessories	Tubes Holder

DIAGENODE RELATED PRODUCTS

Kits:

Chromatin shearing

Our Shearing methods provide you with easy and highly reproducible chromatin shearing protocols which are:

- The first key for a successful ChIP
- Fast, optimized and user-friendly standardised for better reproducibility and as a consequence, data analysis is made easier

For rapid global chromatin extraction

Shearing ChIP kit, LowCell kit	kch-805-003	for 200.000 to 2.106 cells (depending on # cells/IP)

For 'sensible' Nuclear extraction

Shearing ChIP kit , Red Kit	kch-redmod-100	for up to 100.10^6 cells (depending on # cells/IP)
Shearing ChIP kit , Red Kit	kch-redmod-400	for up to 400.106 cells (depending on # cells/IP)

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP). ChIP assay offers a huge potential to boost knowledge about the regulation of the genome expression. Used in a large number of life science disciplines and addressing several essential questions like cellular differentiation, tumour suppressor gene silencing,...

ChIP, ChIP-on-chip and ChIP Seq are used to investigate interactions between proteins and DNA in vivo. It allows the identification of binding sites of DNA-binding proteins in a very efficient and scalable way for proteins generally operating in the context of chromatin like transcription factors, replication-related proteins, histones, their variants, and histone modifications.

All our products have been extensively validated in ChIP on various targets. The combination of all our Quality Controlled kits, reagents and equipment is the perfect starting point to your success.

	Transcription ChIP kit	Histone ChIP kit	OneDay ChIP kit	LowCell# ChIP kit
Optimized for	Transcription factors and co-factors studies	Histones & modified histones	All DNA-protein interaction	All DNA-protein interaction
Ideal for	Traditional use	Traditional use	Intensive use	Intensive use / rare material
Suitable for ChIP-on-chip	yes	yes	yes	yes
Amount of cells/IP	10e6	10e5	10e5 - 2 10e6	10e3 - 10e4
Time from cell collection to PCR	3 days	3 days	1 or 2 days	1 or 2 days
Buffers & Reagents for	Cell fixation - collection - lysis / Shearing / IP / DNA purif	Cell fixation - collection - lysis / Shearing / IP / DNA purif	IP / DNA purif	Cell fixation - collection - lysis / IP / DNA purif
Control Antibodies	anti-TBP or RNA Pol II / anti IgG (rabbit or mouse)	anti H3 (K4me3) / anti IgG (rabbit or mouse)	anti IgG (rabbit or mouse)	anti IgG (rabbit or mouse)
Control PCR primers pairs	GAPDH / idem -0.5kb / idem -1kb / c-fos / beta actin / myoglobin exon 2	BMX / c-fos / beta actin / myoglobin exon 2	-	SAT2 / c-fos / beta actin / myoglobin exon 2
Specific Key feature	Traditional, lots of publications, comprehensive,...	Traditional, lots of publications, comprehensive,...	Quick, one buffer, adaptable, to all kind of studies, no phenol: chloro,...	Quick, low amount of material / IP, sensitive, one buffer, adaptable to all kind of studies, no Phenol: chloro,...
#IP per kit	18	18	60 or 180	16

DNA methylation

During DNA replication, methylation is one of the post-synthesis modifications. It has been proven to be manifested in a number of biological processes such as regulation of imprinted genes, X chromosome inactivation, tumour suppressor gene silencing in cancerous cells and also acts as a protection mechanism adopted by pathogen DNA. Methylation usually occurs in the CpG islands, a CG rich region, upstream of the promoter region.

Diagenode proposes two complementary methods for the study of DNA methylation patterns: Methylated **DNA Immunoprecipitation** and **Bisulfite conversion**.

MeDIP kit™: The Novel Diagenode Methyl kit is designed to immunoprecipitate methylated DNA (Methyl DNA IP).
This brand new Methyl DNA IP method provides you with methylated DNA (meDNA) and unmethylated DNA (unDNA) controls to be used together with your DNA sample allowing direct correlation between IP'd material and methylation status.
This methylation analysis is highly specific and each IP is quality controlled: essential keys for reliable results.
The kit is:

- Specific and quantitative (several internal controls)
- Robust and simple (all in one tube)
- Fast (less than 3 days)
- User-friendly (comprehensive manual and quick start)

In addition, the **MeDIP kit™** is validated for sample preparation prior to **MeDIP-on-chip** and **MeDIP-seq** assays.
One format: 10 IPs per kit. (cat. #: mc-green-001)

MethylEasy™

- **Xceed** (cat # MEA-BISXCE-040): Rapid, ultra-sensitive, 40 reactions
- **Original** Bisulphite Kit (cat # MEA-BISLPH-025): Original precipitation based kit, 25 reactions
- **High Throughput** for Centrifuge (cat # MEA-BISWEC-096c): Original method, 96 well format for centrifuge, any centrifuge that will accommodate a height of 5.1cm (2.0in)
- **High Throughput** for Vacuum (cat # MEA-BISWEC-096c): Original method, 96 well format for Vacuum Manifold.

Original MethylEasy™	MethylEasy™ Xceed
Min 1ng of DNA	Min 50 pg of DNA
Denature DNA with NaOH and incubate for 15 minutes	Idem
Add conversion reagents and incubate for 4 - 16 hours	Add conversion reagents and incubate for 45 mins
Precipitate and wash the DNA, 45 to 75 minutes	Purify the DNA via column for 10 mins
Resuspend the DNA and desulphonate for 30 mins to 1 hour	Desulphonate for 20 mins
Full conversion 6 to 18 hours total time	Full conversion 90 minutes total time

Antibodies:

• Selection of our Antibodies

We achieve our selection of our Antibodies through:

• Collaborators and customer suggestions (licensing)
• Research focus areas
• Conferences

We make our own antibodies and we source antibodies from other suppliers (academic laboratories, primary manufacturers).

• Antibodies QC

We make every effort to offer our customers the best antibodies regardless of their source.

We struggle to characterize our antibodies regarding different applications, target specificity and species cross-reactivity. Our policy is to follow the most stringent testing conditions and to publish all information on our website.

Example of flowchart of an antibody production and QC:

• Peptide design in house, looking for the best immunogenicity for rabbit polyclonal abs production
• Peptide synthesis following the most stringent quality criteria
• Rabbit immunization: Rabbits are immunized with 200 microgram's of peptide conjugated to KLH.
    - Pre-immune sampling as negative control for later testing immune response.
    - Rabbits are immunized at Day 14, 28, 56.
    - Bleeds at Day 38, 66 and final bleed at Day 87 (unless we decide prolongation).
• For each intermediate bleeds, we test the immune response and cross reactions in order to monitor the immunization program.
• QC tests are applied successively on crude sera and after affinity purification.
• A technical adjustment can occurs as well as simple rejection and renewal of the complete program when the QC standards are not reached
•After initial testing in our laboratory, we send the product in the labs of one of our expert collaborators or customer for further testing. We recompense all published feedback. This information always included into the antibody's datasheet section.

Noise level of Bioruptor measured in Accredited Acoustics Laboratory

Leq = noise level in dBA = 78.4 dB
Lmax = dB Peak = 87.6 dB
There isn't any CEE noise data applicable to the same type of device like Bioruptor (ultrasounds emitter). It seems that nothing has been done about that so far.
With Bioruptor's values, we truly respect the English and Belgian work regulations which are:

1° Exposure Limit Value:
The exposure limit value is the maximum amount of noise an employee may be exposed to one any single day (8 hours long). It represents a high risk above which employees should not be exposed.
LEXPOSURE, 8h = 87 dB(A)
PPEAK = 200 Pa witch means 140 dB(C) referring to 20 µPa

2° Upper Exposure Action Value:
The exposure action value is a daily amount of noise exposure above which employers are required to take action to control exposure.
LEXPOSURE, 8h = 85 dB(A)
PPEAK = 140 Pa witch means 137 dB(C) referring to 20 µPa

3° Lower Exposure Action Value:
LEXPOSURE, 8h = 80 dB(A)
PPEAK = 112 Pa witch means 135 dB(C) referring to 20 µPa
LEXPOSURE, 8h values are limit values of exposure when you use Bioruptor for 8 hours a day, which you don't. So, these values have to be corrected if time of exposure is shorter. For example, Lower Exposure Action Value for 15 minutes (LEXPOSURE, 15 min) is 95 dBA.

So, legal limits of noise exposure are not exceeded according to Belgian and English work regulations. However Bioruptor's sound can be annoying, therefore we suggest the use of hearing protection.

Use of Bioruptor by pregnant women

The Bioruptor is in accordance with European norms regarding noise nuisances. We have been testing it extensively at Diagenode and results are satisfactory. We recommend anyway isolating the Bioruptor as much as possible to avoid the noise inconvenient. Iex in a closed room dedicated to instruments only or either in a cold room. Like any other type of high frequency sonication equipment, the Bioruptor must be used with all protection recommended: noise box closed, holding ear protection.

We recommend that pregnant laboratory workers (or any user, in case of doubt), should discuss the work they perform and the fact that they handle a Bioruptor with their personal physicians to determine what, if any, work restrictions are needed. Any restrictions placed by the physician should be discussed with the laboratory's PI and Hygiene Officer immediately.
Without medical advice, Diagenode recommend that the use the Bioruptor by pregnant women get curtailed for the duration of the pregnancy.

The water bath unit which is the ultrasounds emitter contains fragile mechanisms. Be as careful as possible when you handle it. When you need to empty the water tank, use the PLASTIC PUMP provided with the machine. Try not to move the unit to pour out water.

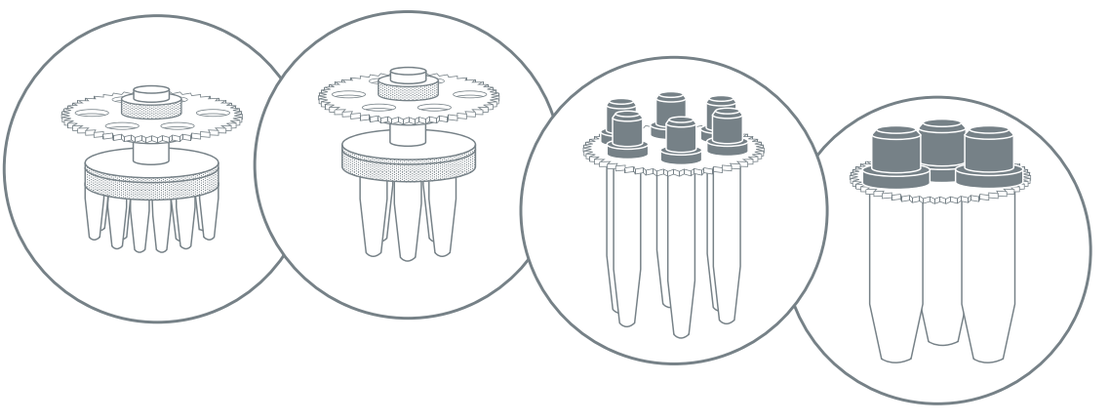
Moreover, when you use the machine the water temperature must be constantly monitored. The sonication phenomenon transfers energy into the water bath producing an increase in temperature. Do not allow water temperature to exceed 40°Celcius (105° Fahrenheit) or damage will occur. Therefore, Diagenode recommends either changing the water as often as possible with 4°C (40° Fahrenheit) or purchase RTE- 7 water cooler system (BioAcc-cool). Do not turn the machine more than 20 minutes without supervision.

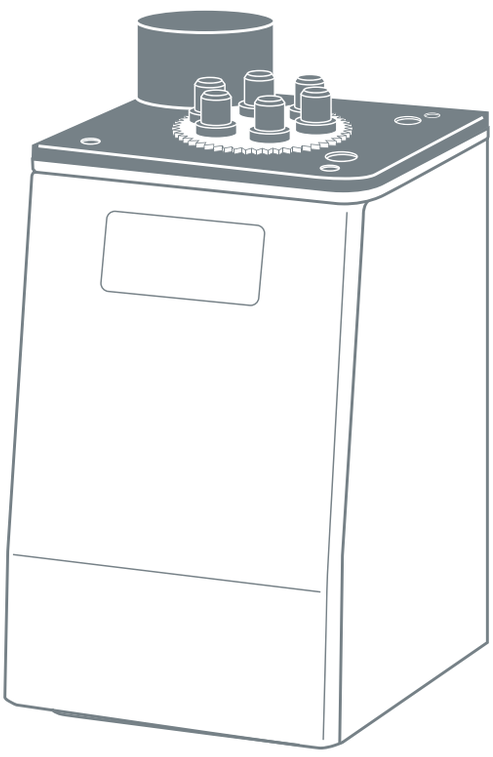
Always:

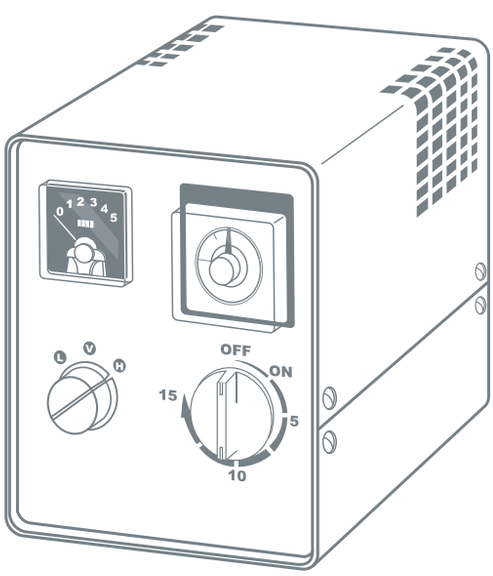
1. Do not turn on the machine without water

2. Respect and watch the water level. Deviation from water line may cause damage.

3. Use 15 ml adaptor with the aluminium o-ring or scratches will occur on the tank bottom.







Instruction Manual (version 01)

Diagenode sa
CHU, Tour GIGA, 3e étage
Avenue de l'hôpital, 1
4000 Liège - BELGIUM
Tel. +32 (0)4 364 20 50
info@diagenode.com
www.diagenode.com

diagenode