diagenode

A Hologic Company

Bioruptor® Plus

Sonication System

Cat. No. B01020001 - B01020002- B01020003

USER GUIDE

Version 5 03_2022

Guarantee

Limited two years global warranty

Diagenode guarantees all products from any manufacturing defects as we rigorously test all products to meet strict quality standards. Diagenode warrants that all standard components of its instruments will be free of defects in materials and workmanship, unless the original quotation or accompanying documentation states a different warranty period. All warranty periods begin on the date of delivery and apply only to the first purchaser of the product. If a manufacturing defect arises and a valid claim is received within the warranty period, Diagenode, at its discretion, will repair or replace the product in accordance with the warranty terms and conditions stated herein. In case of repair or replacement of a product under warranty, Diagenode will cover the expenses to return the repaired or replacement product.

This warranty covers only manufacturing defects and does not cover any damage caused by misuse, lack of compliance to recommendations stated in the manual, neglect, accidents, abrasion, or exposure to extreme temperatures, chemical solvents, or acids. We strongly recommend that maintenance or repairs of Diagenode's products are performed by our approved Diagenode service center. Improper or incorrectly performed maintenance or repairs will void the warranty.

Technical assistance & ordering information

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https://www.diagenode.com/en/pages/support

Contents

BIORUPTOR® PLUS

Critical Steps for Maintenance and Efficient Shearing

DON'T	DO
✗ Turn on the instrument without water	✓ Change water at least once every month
✗ Exceed 30 min of total sonication (total ON cycle) *	✓ Use deionized or distilled water
✗ Tilt the sonication unit	✓ QC your system with our DNA QC Kit

Water quality

- The water bath must be filled with purified water according to specification (see table) to the fill line. Change water at least every month and clean sonication tank with a soft tissue.

	Grade of Water	Compatibility with the Bioruptor®
Ultrapure water	Type 1 or Type 1+	No
Deionized water	Type 2+ or Type 2	Yes
Distilled water	Type 3	Yes
Tap water	N/A	No

Water bath temperature

- Optimal temperature for sonication is 4°C. Sample temperature should not exceed 10°C.
- Maintain the temperature with Diagenode Water Cooler and Single Cycle Valve. The Water Cooler must be installed below the sonication unit to ensure a correct water flow.

Magnetic Ultrasound Emitter Maintenance

- The ultrasound waves are created from a series of magnets that are attached to the water tank. This system is very sensitive to the heat generated during a run.
- Do not exceed 30 min* of total sonication per run (total ON cycle). Set OFF cycle time higher or equal to ON cycle period. It is critical that the machine is allowed to cool at least 15 min between runs. Damage resulting from noncompliance to manual instructions will void the warranty and shorten the lifespan of the machine.
- Ultrasound Emitters can be damaged by tilting or jarring the machine. Exercise care if moving water tank.

** It can be increased to 45 min using the low power mode on the Bioruptor® Plus*

Validated tubes for the Bioruptor® Plus

- DNA shearing: 0.5 ml Bioruptor® Microtubes (Cat. No. C30010013).
- Chromatin Shearing: 1.5 ml TPX Microtubes (Cat. No. C30010010) and 15 ml TPX tubes (Cat. No. C30010009). Others tubes might be used but will require additional optimization. Once a brand of tube is optimized, switching brands may result in changes in sonication efficiency.

Fitting 0.5 ml or 1.5 ml tubes in the corresponding tube holder

1. Place the tubes on the corresponding tube holder (0.5 ml tube holder - Cat. No. B01200043 or 1.5 ml tube holder - Cat. No. B01200011). Never leave empty spaces in the tube holder. Fill the empty spaces with tubes containing the same volume of water. Screw the lid on the tube holder without over-tightening the lid.
2. Carefully place the tube holder on the holding plate.
3. During sonication, samples must remain at the bottom of the tube. If needed, briefly centrifuge samples during sonication after pausing the run.

Fitting of 15 or 50 ml tubes in the corresponding tube holder

1. Loosen both the blue and the black top prior to placing the metallic reflecting bar in the tube.
2. First tighten the blue ring then the black top. This will ensure the O-ring is properly placed in the tube.

Introduction

Diagenode's Bioruptor® Plus uses a gentle method of sonication to retain the integrity of DNA and/or biological complexes, including chromatin, protein-protein binding, protein-DNA complexes and other biochemical and biological assay systems. The Bioruptor® Sonication System uses a sonication bath to generate indirect sonication waves, which emanate from an ultrasound element below the water tank. Because the system is gentler than other sonicators, the Bioruptor® produces better and more consistent results than with harsher sonication methods. Up to 12 closed tubes can be sonicated in parallel and the continuous rotation of tubes allows even distribution of the energy for efficient sonication. The Bioruptor® enables automation of sonication, guaranteeing higher reproducibility of results.

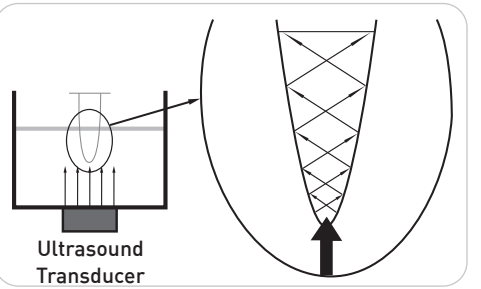


Fig 1. Propagation in 0.5 ml tubes and 1.5 ml tubes

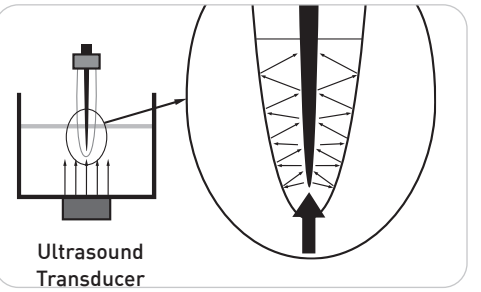


Fig 2. Propagation in 15 ml & 50 ml tubes

The effect of ultrasound on biological samples

The Bioruptor® sonication system uses ultrasound to create focused mechanical stress to lyse cells or shear DNA or chromatin. Ultrasound waves pass through the sample expanding and contracting the liquid. During expansion, negative pressures pull the molecules away from one another to form a cavity or bubble. This process is called cavitation. The bubble continues to absorb energy until it can no longer sustain itself and implodes. This produces intense focused shearing forces, that disperse and break biomolecules. The fragmentation of DNA takes place as a consequence of this mechanical stress or shear.

With the Bioruptor®, the entire volume of water present in the sonication bath is exposed to ultrasound, allowing all the samples to be efficiently sonicated (Figure 1). For larger tubes (15 ml or 50 ml), the transfer of the ultrasound to 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 ultrasound originated from the sonication bath and improves the sample sonication efficiency by a patented resonance system (Figure 2).

Noise level measurements & precautions

CEE noise data is not applicable to the Bioruptor's ultrasound emitter as no tests have been conducted on similar devices to date. See listed noise levels (as measured in the Accredited Acoustics Laboratory) and precautions for the Bioruptor®:

L_{eq} = noise level in dBA = 78.4 dB
L_{max} = dB Peak = 87.6 dB

1. Exposure limit value

The exposure limit value is the maximum amount of noise an employee may be exposed to on any single day (8 hours). Exposure beyond this limit presents a high risk to the user.

$L_{EXPOSURE}$, 8h = 87 dB(A)

P_{PEAK} = 200 Pa (140 dB(C) referring to 20 µPa)

2. Upper exposure Action value

The exposure action value is the upper daily limit of noise exposure. Exposure beyond this value requires employers to take action to limit user exposure.

$L_{EXPOSURE}$, 8h = 85 dB(A)

P_{PEAK} = 140 Pa (137 dB(C) referring to 20 µPa)

3. Lower exposure action value

$L_{EXPOSURE}$, 8h = 80 dB(A)

P_{PEAK} = 112 Pa (135 dB(C) referring to 20 µPa)

Use of Bioruptor® by pregnant women

Exposure to 20-60 kHz sound waves has not been shown to be harmful to human health. However, we would recommend avoiding unnecessary exposure. Diagenode recommends that pregnant women should not be exposed to 20-60 kHz wave lengths for a long period of time.

Bioruptor® Technical Specifications

BIORUPTOR®	
Power Supply	100 - 240 Vac
Control unit dimensions	350(W) x 260(D) x 165(H) mm
Sonication unit dimensions (sonication bath)	175(W) x 160(D) x 265(H) mm
Soundproof box dimensions	350(W) x 350(D) x 500(H) mm
Sonication bath volume	750 ml
Timer	Digital
Possibility to control water flow via connector kit for water cooler	Yes
Interval Timer	0-60 sec. or min. (on/off cycles)
Total Weight	30 Kg
Tube holder	Available for 0.5 - 1.5 - 10 - 15 & 50 ml tubes
Number of samples to be processed simultaneously	0.5 ml tubes – 12 1.5 ml tubes - 6 10 ml tubes - 6 15 ml tubes - 6 50 ml tubes - 3

Getting to know your Bioruptor Plus system

Bioruptor Pico components overview


Control Unit


Sonication bath


Motorized lid


Soundproof Box


Power Cable


Control Unit Cable


Tube holder example


Single cycle valve

Sonication bath

The sonication bath is a critical component of the instrument. The generators below the tank produce ultrasonic waves which are then transferred through water. The sonication bath requires special handling and care as described below.

Handling

The sonication bath must remain upright at all times, especially when moved. Tilting the sonication bath or handling roughly may damage the ultrasound emitter component, resulting in a substantial drop in sonication efficiency. If transportation of the apparatus is required after initial set-up, it is imperative to keep the tank at a right angle to the ground during the transport at all times.

Water quality

The water bath must be filled with purified water according to specification (see table page 4) to the fill line. Change water at least every month and clean sonication tank with a soft tissue.

Water temperature

The water in the sonication bath must be kept at 4°C. Ultrasonic waves produced by the Bioruptor Pico generate heat. Drop off in sonication efficiency will occur above 8°C. To ensure preservation of the samples and to prevent damage to the instrument, it is necessary to start the sonication process with cold water and to keep it at 4°C during the sonication process.

Automatic temperature control

The Water Cooler (Cat. No. B02010002, 230 V and B02010003, 115 V) is used to guarantee the automatic temperature control of the sonication bath during the entire sonication process. An additional regulating

valve ensures that the water will only be replaced during the off cycle to avoid any interference between water flow and the sonication process.

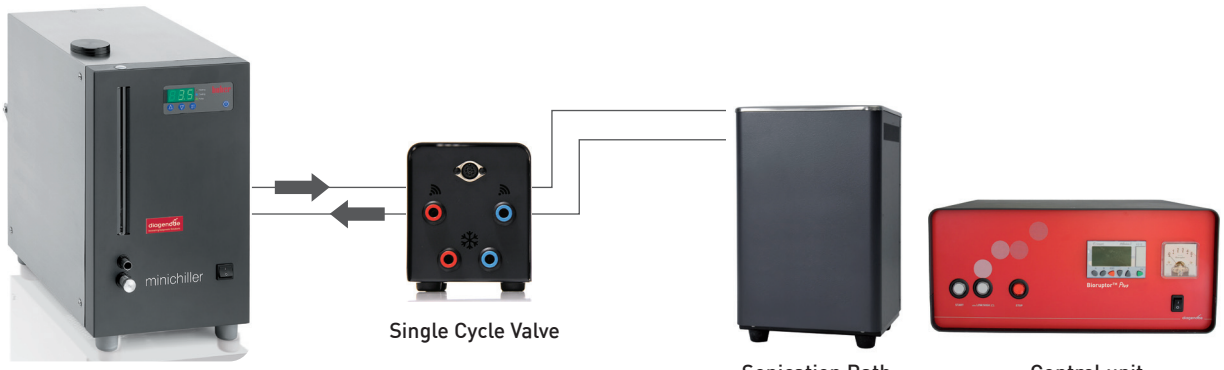


Fig. Setup of the Bioruptor® Plus in combination with the Water Cooler.

Motorized lid

The motorized lid, along with the gear plate accessory, keeps the sample tubes in constant rotation and ensures optimal position in the sonication bath during sonication. When in motion, do not hamper the rotation of the gear plate. Avoid the immersion of the motor into the water. Do not heat the plastic as it will warp.

Metallic Soundproof Box

This metallic soundproof box absorbs more than 30 dBA generated by the ultrasonic sonication bath.

Soundproof Box

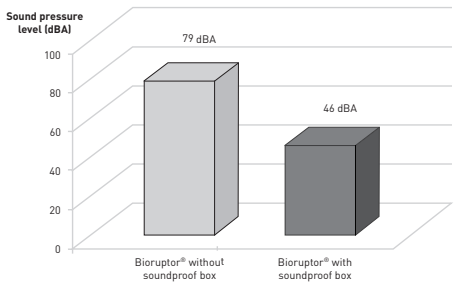
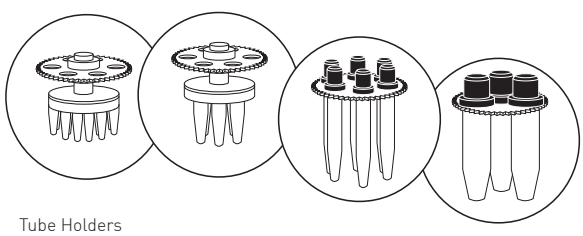


Fig. Sound pressure of the Bioruptor® without and with metallic soundproof box has been measured in a soundproof room.

Tube Holders

Several sizes of tubes can be used with the Bioruptor® Plus. The minimum and maximum sample volume to be used with each container is given in the table below. The 0.5 ml and 1.5 ml tubes can be simply closed and installed in the rotor. For the sonication of larger volumes (10 ml, 15 ml and 50 ml tubes), a stopper with a m
proc
nance efficiency.



Tube Holders

Tube Size	Minimum	Maximum
0.5 ml	50 µl	100 µl
1.5 ml	100 µl	300 µl
10 ml	500 µl	2 ml
15 ml	500 µl	2 ml
50 ml	3 ml	20 ml

Equipment Installation

The following pages contain information on installing your Bioruptor® Plus model. This equipment must only be installed by personnel after reading this section. Consider all hazards even though no particular hazards have been identified during installation. Before starting installation work, turn the main switch off (back side of the control unit) and secure the unit against being re-energized. No special tools are required. Three (3) square meters are needed to set-up the Bioruptor®.

Devices are designed to be safe under the following conditions:

- Indoor use
- Altitude up to 2,000 meters
- Operating external temperature 0°C to 25°C
- Maximum relative humidity 80%
- Transient overvoltage typically present on the MAINS supply
- Degree of protection: IP20
- Power plug must be grounded
- POLLUTION DEGREE 2 (Normally only non-conductive pollution occurs. However, occasionally a temporary conductivity caused by condensation is expected)
- Never install this equipment in a place where environmental conditions and warnings mentioned above are infringed

Installation overview

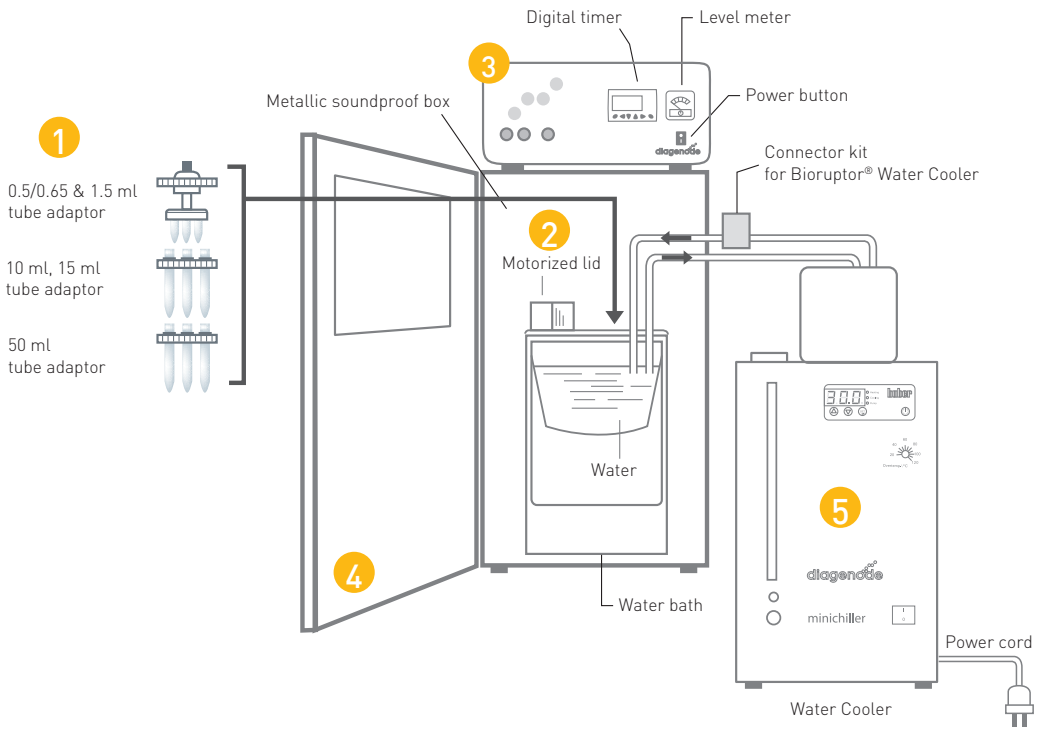
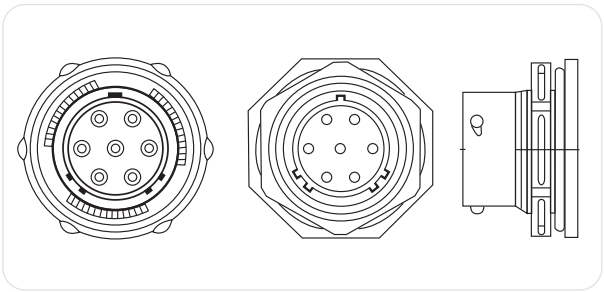


Fig. Schematic installation overview of the Bioruptor® Plus System in combination with the Bioruptor® Water Cooler.

Attaching Cables

The power supply adapter must be plugged into the power grid. An IEC lead is provided.

IMPORTANT WARNING: Ensure that your power inlet (behind the power supply adapter) shows the right voltage corresponding to your area. Otherwise, switch it using a narrow blade screwdriver.



When connecting cables, always be sure pins are properly aligned. Note the indexing pins on the control unit cables for precise mating alignment.

Keep dot facing up.

Once plugged in, secure by turning 90° clockwise until a click is heard.

1 Flexible format

Fits into current workflow with standard tubes.
Scales with flexible sample volume.

2 Rotation

Prevents contamination with closed tubes.
Continuous rotation through sonication bath guarantees equal distribution of energy.

3 Flexible control

Easy to program.
Power range effectively disrupts samples.

4 Gentle ultrasound

Gentle ultrasound method preserves sample.

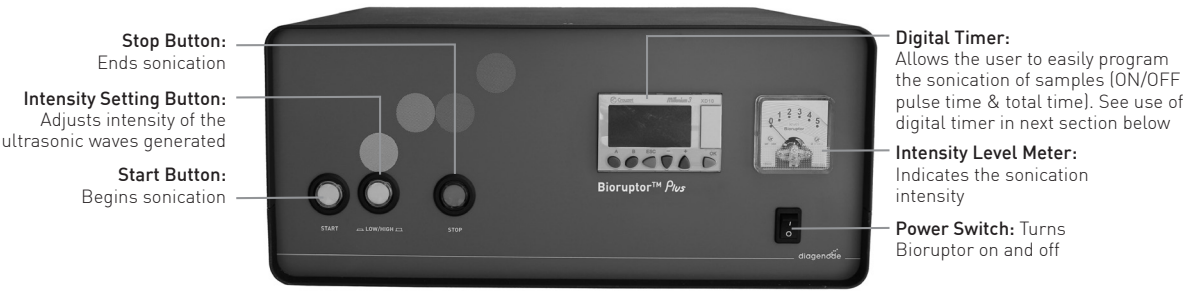
5 Cooling

Cooling system maintains integrity of sensitive samples.

SCAN ME
to get the video of
Bioruptor Plus installation

Controlling the Sonication

CYCLE NUMBER, TIME ON and **TIME OFF** and **SONICATION INTENSITY** are the parameters controlling the sonication.

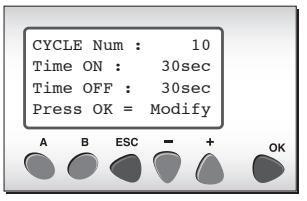


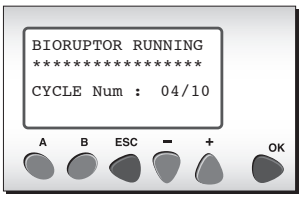
First press + or - depending on the value to be modified. The five flashing black squares move up or down. Once you have selected the parameter to be modified, press OK again. The five flashing black squares disappear and 2 digits start flashing. The digits can be changed by pressing + or -. To select and save the correct number, press OK to confirm or ESC to escape without saving the change.

IMPORTANT NOTE: Minimum time of the off cycle: 30 seconds.

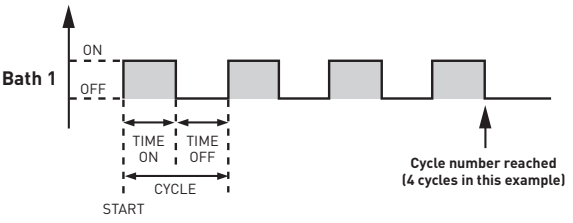
Digital Timer

After the introductory message, the control screen shows the main sonication parameters in the first three lines (CYCLE Number, Time ON, Time OFF) and summary of actions in the last line (see example below).





The display shows cycle 4 of 10.



Bioruptor® NGS will sonicate as shown.

Buttons and their functions

Button A: Pause / restart after pausing

Button B: Press and hold this button during sonication to display T1 (total on time per cycle) and T2 (total off time per cycle).

ESC: Cancels previous selection

OK: Validate selection

- : Decrease selected parameter/move down

+ : Increase selected parameter/move up

Once all parameters are selected and confirmed, press START.

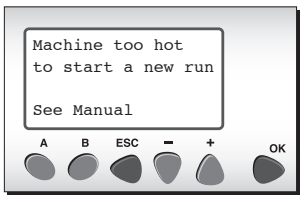
Once the run is started, "BIORUPTOR RUNNING" is displayed on the control screen.

Overheat Shutdown

The ultrasound source is sensitive to high temperatures which means that increases in temperature diminish the sonication efficiency and can damage the device. The production of ultrasound generates heat which is transferred into the sonication bath. To avoid excessive overheating of the ultrasound source, the Bioruptor® Plus (UCD-300) contains a temperature sensor. We recommend that the water in the sonication bath remains between 4˚C–10˚C (39°F—50°F) for optimum sonication.

Note: The sensor does not measure the water temperature inside the ultrasound bath where the samples are submerged.Once a critical limit is reached a warning is displayed on the control screen. Depending on the situation, different screens pop up as described below:

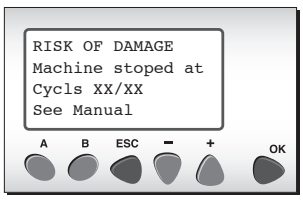
1. **If START is pressed and the instrument stops after a couple of seconds showing the following screen blinking, the instrument could not start a new run due to the ultrasound source's temperature.**



Causes:
- Instrument has been used several times in a row recently without breaks
- Instrument is stored in a place exposed to direct sun
- Room temperature is too hot

What to do:
- Store the instrument in another place if the current one does not meet the installation specific requirements
- Allow for longer breaks between uses
- Place crushed ice in sonication bath to help the cool ultrasound source

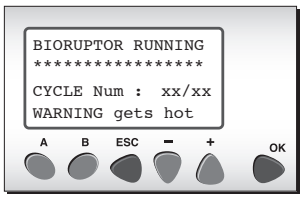
2. **During a run, if the instrument stops and the following screen pops up, the critical temperature has been reached. To protect the ultrasound source from damage, the instrument has stopped and is standing by to be restarted after the ultrasound source has cooled.**



Causes:
- Instrument has been running too long without breaks

What to do:
- Store the instrument in another place if the current one does not meet the installation specific requirements
- Allow for longer breaks between uses
- Place crushed ice in sonication bath to help to cool down the ultrasound source

3. **If the warning message appears at the bottom of the BIORUPTOR RUNNING screen (see below), it means that the critical temperature is about to be reached.**



Causes:
- Instrument has been running too long without breaks

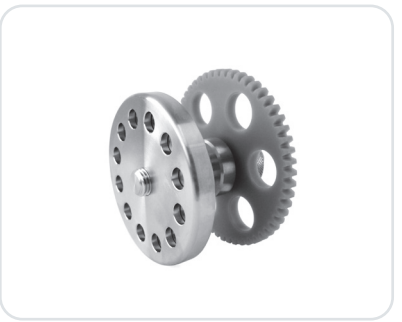
What to do:
- Reduce the number of consecutive cycles or split protocols into two runs separated by a break
- Keep cold water in the sonication bath while running to help keep the ultrasound source at low temperature

Tube holders & tubes

Tube holders - Available for 0.5 ml, 1.5 ml, 10 ml, 15 ml, and 50 ml

To use adaptor tube unit, remove the lower part of the microtube holder by turning counterclockwise. Then place microtubes in the unit. Attach the lower part to the upper part of the adaptor. To guarantee homogeneity of chromatin shearing, the tube holders should always be completely filled with tubes. To ensure reproducibility, always use the same brand of tubes.

- The 2 ml polypropylene tubes (thin-walled) should not be used with the Bioruptor®. 1.5 ml tubes in TPX plastic that provide better ultrasound transfer rates and more efficient sonication are available from Diagenode (see price list).
- Any 0.5 ml or 1.5 ml tube can be used although shearing efficiency is increased by using the hard plastic tubes (TPX hard plastic) available from Diagenode. See catalog numbers on price list.
- For DNA shearing only 0.5 ml Bioruptor® Microtubes (Cat. No. C30010013) can be used.
- The complete adaptor (including O-ring) can be sterilized in the autoclave. After more than 20 autoclave sterilizations, the O-ring might need to be replaced (see price list for spare parts).



0.5 ml tube holder
(Cat. No. B01200043)

1.5 ml tube holder
(Cat. No. B01200011)

10 ml tube holder
(Cat. No. B01200012)

15 ml tube holder (Cat. No. B01200013)

The tube holder for 15 ml tubes is compatible with Diagenode TPX and Falcon tubes. If you use another brand of tubes, you need to check their compatibility. 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 holder (Cat. No. B01200014)

The tube holder for 50 ml tubes is available for Falcon tubes. If you use another brand of tubes, you need to check their compatibility.

Holding Plates (included with any tube holder)

The holding plate for 10 ml and 15 ml tubes can accommodate up to 6 tubes. For 50 ml tubes, the sample holding plate can accommodate up to 3 tubes. The holding plates should always be completely filled to guarantee homogeneity of shearing.

O-rings (for 10 ml, 15 ml, 50 ml tube holders)

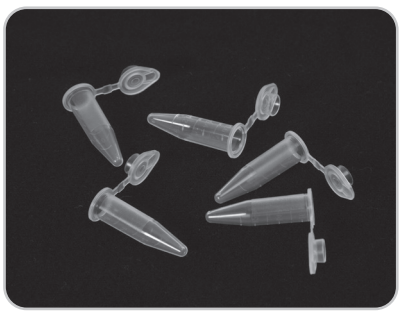
By removing the black knob, it is possible to replace the O-ring. The complete tube holder chip (including O-ring) can be sterilized in the autoclave. After more than 20 autoclave sterilizations the O-ring might need to be replaced (visit www.diagenode.com).

Standard protocols

DNA shearing

For DNA shearing we highly recommend to use the tube holder for 0.5/0.65 ml tubes (Cat. No. B01200043) and the corresponding Bioruptor® 0.5 ml Microtubes for DNA Shearing (Cat. No. C30010013).

0.5 ml tube holder (Cat. No. B01200043)



Bioruptor® 0.5 ml Microtubes for DNA Shearing (Cat. No. C30010013)

To use the tube holder, remove the lower part by turning counterclockwise. Then place microtubes into the unit. Attach the lower part to the upper part of the adaptor. To guarantee homogeneity of DNA shearing, the tube holders should always be completely filled with tubes. Never leave empty spaces in the tube holder. Fill the empty spaces with tubes containing the same volume of distilled water.

Operating conditions

Sample volume: 100 µl

Tubes: Bioruptor® 0.5 ml Microtubes for DNA Shearing (Cat. No. C30010013)

Tube holder: 0.5 ml tube holder (Cat. No. B0120010) for 12 x 0.5 ml tubes

Sonication buffer: TE (10 mM Tris, 1mM EDTA), pH 7.5 - 8.0

DNA concentration: 0.001-0.02 µg/µl (0.01 µg/µl recommended)
Samples are vortexed (10-15 sec) and centrifuged (10 sec) before shearing.

For optimal results samples should be stored on ice during 10-15 min prior to sonication.

Temperature: 4°C

Power setting: L position (Low)

Sonication cycle and sonication time: varies depending on desired DNA size (see table)

Note: Recommended protocols are subject to change without notice. Additional protocols are available on demand.

Target size	Cycle conditions (On/Off times in sec.)	Number of cycles
1250 bp	15/90	2 cycles
950 bp	15/90	4 cycles
750 bp	30/90	3 cycles
550 bp	30/90	5 cycles
400 bp	30/90	6 cycles
350 bp	30/90	8 cycles
300 bp	30/90	10 cycles
250 bp	30/90	15 cycles
200 bp	30/90	30 cycles
150 bp	30/30	70 cycles

The protocol settings listed above are recommended guidelines and actual results may vary depending on the type and amount of starting material, purity level, concentration and/or sample viscosity. It is highly recommended that a time course response experiment be carried out (e.g. varying the time of "On" and "Off" durations as well as the number of cycles) to determine the appropriate treatment for your specific sample. Starting material with a smaller sample volume and a greater concentration than the recommended range may require a different time course to ensure homogenous shearing results.

Important comments about DNA shearing

The Diagenode ACT (Adaptative Cavitation Transfer technology) process is highly reproducible, however attention must be paid to the following treatment attributes to ensure best results:

- **Tubes:** At present, the recommended tube vessels are the Diagenode's Bioruptor® 0.5 ml Microtubes for DNA Shearing (Cat No. C30010013). Pay attention not to damage the cap when closing the tubes since this could alter sonication results.

- **Sample volume:** The recommended volume of the Diagenode's Bioruptor® 0.5 ml Microtubes for DNA Shearing (Cat No. C30010013) is 100 µl. When using lower volumes (eg. ≤ 50 µl), less reproducible results may be observed due to an alteration of the ultrasonic waves distribution in the sample fluid; thus, reducing the efficiency of sonication which may result in broader size distribution or larger peaks.

- **Sample concentration:** Diagenode recommends using a DNA concentration ranging between 1 and 20 ng/µl (10 ng/µl recommended). Using larger concentration (eg. 50-100 ng/µl) may result in broader peaks or variable peak distribution.

- **Sample preparation:** Sample viscosity may have a major impact on sonication results. Careful resuspension of DNA sample is strongly recommended before sonication processing. Multiple pipetting and gentle vortexing followed by a short centrifugation to recover sample volume at the bottom of the tube is therefore strongly recommended. Storing DNA samples on ice during 10-15 min before sonication has also been shown to improve reproducibility.

- **DNA Qualtity:** DNA quality and quantity must be considered carefully since bad quality and quantity DNA may have several impacts on sonication and next-gen sequencing downstream applications. First, DNA contamination (eg. from superfluous nucleic acids such as RNA, small nucleic acid fragments, excess proteins, or other contaminating materials) may interfere with DNA measurement method leading to incorrect DNA quantitation thus. Also contaminating RNA in genomic DNA preparation might generate a biased fragment distribution profile on microfluidics-based platform (eg. Agilent Bioanalyzer) or alter sonication effiency.

  Therefore it is highly recommended to use only high quality DNA when sonicating DNA for next-gen sequencing library preparation. The DNA sample to be processed should be highly pure, having an OD260/280 ratio of between 1.8 and 2.0, and should be as intact as possible. DNA extracted using standard techniques (eg. Proteinase K digested, double phenol/chloform extraction, ethanol precipitated, treatment with RNase-DNase free enzymatic digestion to remove contaminant RNA) or commercial spin-column based kits are recommended.

- **Water temperature:** Propagation of ultrasound in a liquid unavoidably produces heat that can ultimately alter DNA sample (e.g. by thermal denaturation). To ensure the best preservation of the sample, it is recommended to start the sonication process with the water cooler set on 4°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 replace the "pre-cooling" step described above.

- **Automatic temperature control:** A recirculating Water cooler is used to guarantee the automatic temperature control of the sonication bath during the whole sonication process. This Water cooler Cat No.B02010002, 230 V and B02010003, 115 V produces a regular water flow with a constant water level in the tank.

- **Sonication time:** Minor adjustments in cycle number may be made to optimize results for various sample types and concentrations. The table above listing the cycle parameters and numbers is a recommended guideline. Actual results may vary depending on the amount and type of starting material, concentration, viscosity and/or plastic tubes. Diagenode recommends setting up a time dose response experiment for determining appropriate cycle number. Larger length starting material (e.g. total genomic DNA) and higher concentration may require a longer dose to ensure a homogeneous shearing result.

- **Sonication bath:** The sonication bath is a critical component of the Bioruptor® sonication system.

1. **Water purity:** Contaminants such as algae and particules may alter the ultrasonic waves propagation, resulting in broader size distribution or larger peaks. Bath water should be pure distilled water, changed regularly.
2. **Sonication bath maintenance:** The sonication bath metal surface is fragile and requires a careful maintenance. Use only soft sponge and distilled water to remove traces. Never use scratch scrub sponge since this would alter the ultrasonic wave emitter surface.

Supplementary Data

Please note that there are three main sources of variation in both peak base-pair size and distribution:

1) The physical process of DNA fragmentation might not be entirely random in AT- or GC- rich regions.

2) The analytical process to determine fragment size has inherent variances (for example, gel electrophoresis and microfluidics-based platform). Therefore, fragment distributions and peak values, even from technical replicates, may not appear identical. If the sheared DNA sample will be resin or column purified or concentrated prior to analysis, please remember to take out an aliquot for use as control prior to that step. Column purification and concentration of the sheared DNA will generate a biased fragment distribution profile due to the inherent greater loss of the smaller DNA fragments.

3) RNA contamination in genomic DNA preparation should be carefully removed using RNase-DNase free enzymatic digestion since they might generate a biased fragment distribution profile on microfluidics-based platform (eg Agilent Bioanalyzer) or alter sonication effiency.

Chromatin shearing

Critical points for chromatin shearing

- Chromatin shearing efficiency varies on cell type. **Each cell type might need additional protocol optimization.**
- The extent of cross-linking is critical for the efficient disruption of fixed cells and also affects DNA yield and average size of chromatin fragments. Over-cross-linked chromatin will not produce small fragments, even by prolonged sonication. Fix cells for 8-10 min at RT, always stop the reaction by glycine and wash 2-3 times with ice cold PBS.
- Cell density affects the sonication efficiency. Do not use too dense cell suspension. Optimal density is about 1-3x10^6/100 µl of sonication buffer.
- Fresh formaldehyde for fixation.

Shearing of chromatin from adherent cell lines

For the adherent cells, we recommend to first harvest cells by trypsinization and perform chromatin cross-linking in a cell suspension rather than on dishes as it results in a better reproducibility and consistency between experiments.

1. Discard medium to remove dead cells and wash cells by adding cold PBS
2. Harvest cells by trypsinization

3. Transfer cells in a tube containing 10 ml PBS (RT) and centrifuge 5 min at 1.300 rpm. Keep the cell pellet and discard the supernatant. Wash the cells again in PBS

Note: At this step, cells might be counted.

4. Add PBS to a final volume of 500 µl for a **maximum of 10x10^6 cells** (for more cells, perform the fixation in a separate tube)
5. Add formaldehyde to a final concentration of 1%, mix gently and incubate for 8-10 min at RT with rotation
6. Stop the cross-linking reaction by adding glycine to a final concentration 0.125 M and incubate for 5 min at RT with rotation
7. Wash cells 3 times with cold PBS
8. Resuspend cells in an appropriate volume of a Lysis buffer containing SDS (0.7-1%). 1x10^6 -3x10^6 cells/300 µl are recommended for shearing in 1.5 ml tubes. Lyse cells on ice for 5-10 min. Vortex and centrifuge tubes before putting in Bioruptor®

Note: Nuclei isolation is recommended when working with 3x10^6 cells to 10x10^6 cells. (Shearing ChIP kit from Diagenode is available for this purpose, kch-redmod-100). Diagenode 1.5 ml TPX Microtubes are recommended for efficient chromatin shearing (Cat. No. C30010010).

9. Sonicate samples with Bioruptor® Plus with refrigerated sonication bath for 10-20-30 cycles of 30 sec ON and 30 sec OFF at HIGH setting. Briefly vortex and centrifuge tubes after each run of 10 cycles
10. Centrifuge samples at 14,000 rpm for 5 min at 4°C and transfer the supernatant into a new tube. Use an aliquot of sheared chromatin (equivalent of 100.000-500.000 cells) for analysis of shearing: perform a reversal of cross-links and analyze on agarose gel. The remaining chromatin might be kept at -80°C.

Shearing of chromatin from suspension cell lines

Note: Cells growing in suspension culture are known to be difficult to shear. Nuclei extraction is recommended before sonication. **Do not use very dense cell suspension for sonication.**

1. Cross-link chromatin with 1% fresh formaldehyde for 8-10 min at RT
2. Stop the cross-linking reaction by adding glycine to the final concentration 0.125 M for 5 min at RT with gentle rotation
3. Wash cells 3 times with cold PBS
4. Extract cell nuclei and use isolated nuclei for shearing (Shearing ChIP kit from Diagenode is available for this purpose, C010020020)
5. Resuspend nuclei in an appropriate volume of Lysis buffer containing SDS (1%). 1x10^6 – 3x10^6 cells/300 µl are recommended for shearing in 1.5 ml tubes. Lyse nuclei on ice for 5-10 min. Vortex and spin down tubes before putting in Bioruptor®

Note: Diagenode 1.5 ml TPX Microtubes are recommended for efficient chromatin shearing (Cat. No. C30010010).

6. Sonicate samples with Bioruptor® Plus with refrigerated sonication bath for 10-20-30 cycles of 30 sec ON and 30 sec OFF at HIGH setting. Briefly vortex and spin down tubes after each run of 10 cycles
7. Centrifuge samples at 14000 rpm for 5 min at 4°C and transfer the supernatant into a new tube. Centrifuge samples at 14,000 rpm for 5 min at 4°C and transfer the supernatant into a new tube. Use an aliquot of sheared chromatin (equivalent of 100.000-500.000 cells) for analysis of shearing: perform a reversal of cross-links and analyze in agarose gel. The remaining chromatin can be kept at -80°C.

Bacterial Cell Disruption

For cell lysis, we highly recommend using 1.5 ml TPX Microtubes (Cat. No. C30010010) or 10 ml tubes (Cat. No. C30010012) and the corresponding tube holders (Cat. No. B01200043 and B01200012). To guarantee homogeneity of sonication, the tube holders should always be completely filled with tubes.

Operating conditions:

Tubes: 1.5 ml TPX Microtubes or 10 ml tubes

Tube holder: 1.5 ml tube holder (Cat. No. B01200043) or 10 ml tubes holder (Cat. No. B01200012) with reflecting bar

Sample volume: 300 µl for 1.5 ml TPX Microtubes
2 ml for 10 ml tubes

Sonication buffer: PBS with protease inhibitor cocktail

Temperature: Maintain at 4°C by using the Water Cooler (Cat. No. B02010002, 230 V and B02010003, 115 V)

Power setting: H position (High)

Sonication cycle: 30 sec ON, 30 sec OFF

Total sonication time: 10 min for Bioruptor® Standard ou Plus
15 min for Bioruptor XL (discontinued)

Note: *Please note that additional optimization might be required depending on the bacterial strain and growth phase. Gram-positive bacteria are more resistant to sonication than Gram-negative bacteria because of the rigid cell wall. Cells in log phase are less resistant than cells in stationary phase. In order to preserve protein structure and activity, avoid a long sonication.*

Protocol:

1. Collect cells by centrifugation at 1,000 g for 10 min at 4°C
2. Wash twice with cold PBS
3. Resuspend cells in cold PBS to OD600 3.0
4. Transfer cell suspension to sonication tubes. For optimal efficiency, use the recommended sample volume
5. Sonicate at High Power for 10 min (Bioruptor® Standard ou Plus) or 15 min (Bioruptor® XL)
6. Centrifuge at 15.000 rpm for 15 min at 4°C
7. Separate the soluble fraction (supernatant) from the insoluble (pellet)
8. The pellet can be used for extraction of insoluble proteins with a denaturating buffer of choice.

Efficient cell disruption with Bioruptor®

Cell suspensions were sonicated for different periods of time ranging from 5 to 20 min. Two types of tubes were tested: Diagenode's 1.5 ml TPX Microtubes (Cat. No. C30010010) and Diagenode's 10 ml Tubes (Cat. No. C30010012). The efficiency of cell disruption was initially determined by measuring optical density at 600 nm. The results indicated that the number of intact cells decreases rapidly with increasing sonication time. After only 5 min of sonication, a significant number of cells were disrupted (Fig.1). Similar results were observed using the Live/Dead BacLight kit (data not shown) which allows the quantification of live cells with intact membranes and discrimination from cells with damaged membranes. Thus, efficient cell disruption is observed after 5-10 min of sonication.

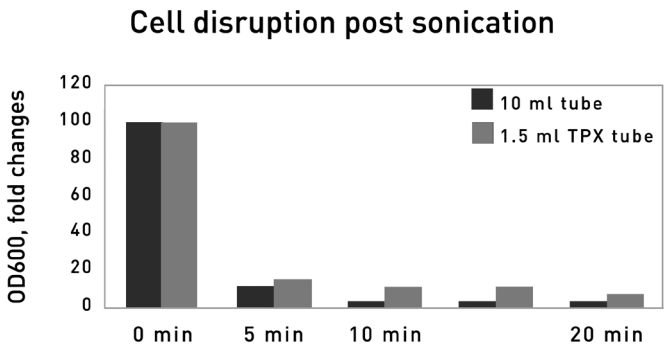


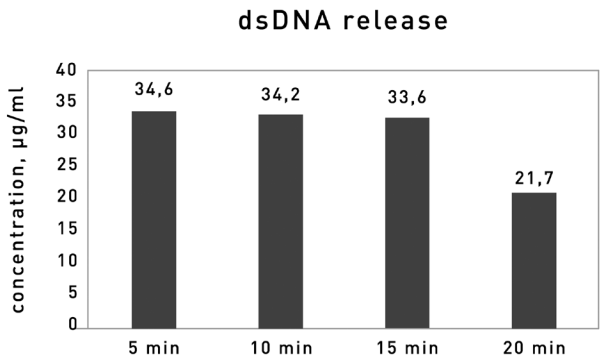
Figure 1: Effect of sonication on cell disruption

The number of intact cells after sonication was determined by measuring optical density at 600 nm. Optical density of the cell culture before sonication (0 min) is arbitrarily set to 100%.

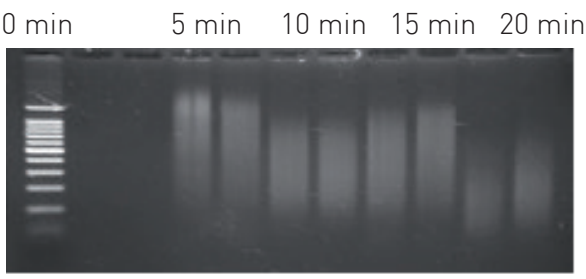
Sheared DNA is released during bacterial sonication

The disruption of bacterial cells by sonication releases DNA with maximum recovery after only 5 min of treatment (Fig.A). The released DNA is fragmented with fragment size dependent on sonication time (Fig.B)

A.



B.



Figures : Effect of sonication on DNA release

Figure A: The DNA concentration in each sample after sonication was quantified with the DNA BR assay kit (Invitrogen)

Figure B: An aliquot of each sample before (0 min) and after sonication was run in a 1.5% agarose gel stained with SybrSafe and visualized in UV light. Lane M represents a 100 bp ladder.

Troubleshooting

Bioruptor: Chromatin Shearing FAQs

Critical Steps	Questions	Answers	Comments
Fixation	What is the formaldehyde final concentration	1%	Correct formaldehyde concentration in fixation is critical.
	How long is the fixation step?	Fix for 10 min (with a time course when needed)	It is possible to fix for as little as 5 min (depending on your protein of interest for subsequent ChIP assays).
	What is the temperature to use for fixation?	Fix at room temperature	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. Use glycine to stop the fixation.	
Cell lysis	How can I achieve complete cell disruption?	Do not use too many cells in the cell lysis buffer. Lyse about 5x 10e6 cells/1 ml	The HighCell # ChIP kit is compatible with cell numbers up to 10 million cells in small volumes.
Number of cells/ shearing buffer volume	What is the amount of cells per shearing trial to use?	1x 10e6–10x 10e6cells/ 300 µl 3x 106–30x 106 cells/ 1 ml	Do not use a too high cell concentration.
Shearing buffer	What is the key buffer component?	Include detergent in buffer	Quality and quantity of detergent is important.
Shearing step	How long is the shearing?	Perform a time course for chromatin shearing	It is possible to shear from 5-30 min. If 30', interrupt sonication after every 10 min and centrifuge tubes briefly before proceeding with the remaining time.
	What is the optimal cycle?	30 seconds "ON" + 30 seconds "OFF"	
	What is the best temperature for shearing?	4°C	Make sure sonication bath is kept cool. Once optimal conditions are reached, use for all assays to assure reproducibility.
	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
Checking for high-quality shearing on an agarose gel	What kind of gel should I use to determine size accuracy?	Check disrupted material on a 1% agarose gel (10 µl/lane). Run the gel slowly	Reverse cross-link from DNA after phenol/chloroform extraction before loading on gel.
	What do smears indicate?	Gel electrophoresis of cross-linked samples often gives smears on gel. Also take several pictures of the gel to assure image quality.	To obtain clearer image with accurate fragment size, reversion of the cross-linking is advised.
	How much DNA should I load and is RNAse treatment necessary?	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 5 µg/lane. Also treat the sample with RNAse.
	What should my running buffer concentration be?	1X TAE or TBE is preferred to 0.5X TAE which can lead to smears on gel.	
	Will using an old gel cause problems?	Use a freshly prepared gel and fresh buffer.	Do not reuse an old gel.

Ordering information

Products	Cat. No. (new)
Bioruptor® Models	
Bioruptor® Plus for 1.5 ml tube holder	B01020001
Bioruptor® Plus for 1,5 & 15 ml tube holder	B01020002
Bioruptor® Plus for 0.65 ml tube holder	B01020003
Cooling System (included)	
Water Cooler	B02010002, 230 V - B02010003, 115 V
Single Cycle Valve for Water Cooler	B02020005
Consumables	
1.5 ml TPX Microtubes	C30010010
15 TPX Tubes	C30010009
0.5 ml Bioruptor® Microtubes	C30010013v
Tube Holders	
0.5 ml tube holder for Bioruptor® Standard, Bioruptor® Plus	B01200043
1.5 ml tube holder for Bioruptor® Standard & Bioruptor® Plus	B01200011
10 ml tube holder for Bioruptor® Standard & Bioruptor® Plus	B01200012
15 ml tube holder for Bioruptor® Standard & Bioruptor® Plus	B01200013
50 ml tube holder for Bioruptor® Standard & Bioruptor® Plus	B01200014