

diagenode

Innovating Epigenetic Solutions

Bioruptor® Sonication System

USER MANUAL

Version 1.5

Bioruptor® NGS

Cat. No. UCD-600



Guarantee

Limited one year 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 for a period of one (1) year from the date that the warranty period begins, 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.

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For a complete listing of Diagenode's international distributors, visit:
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Critical Steps for Maintenance and Efficient Shearing

General warnings



- **DO NOT** turn on the instrument without water.
- **DO NOT** tilt the water bath. To change the water, use either the plastic pump or a beaker.

Water bath levels

- The water bath must be filled with distilled water to the fill line. Fill line replacement stickers can be obtained by contacting Diagenode.
- Change water at least once per week.

Water bath temperature

- Optimal temperature for sonication is 4°C. Sample temperature should not exceed 10°C.
- The Diagenode Water Cooler (Cat. No. BioAcc-Cool) has to be used in combination with the Connector kit for Water Cooler (Bioruptor® Plus & NGS) (Cat. No. VB-100-0001) to guarantee the automatic temperature control of the water bath during the entire sonication process.

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 1 hour of total sonication per run. It is critical that the machine is allowed to cool at least 20 minutes between runs. Damage resulting from non compliance 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.

Validated tubes for the Bioruptor® NGS

- DNA shearing: Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat.No. WA-005-0500).

Fitting 0.65 ml tubes in the tube holder

1. Place the tubes on the corresponding tube holder (0.5/0.65 ml tube holder – Cat. No. UCD-pack 0.5). 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.

Introduction

Diagenode's Bioruptor® NGS uses a gentle method of sonication to retain the integrity of DNA. The Bioruptor® Sonication System uses a water 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.

The effect of ultrasound on biological samples.

The Bioruptor® sonication system uses ultrasound to create focused mechanical stress to shear DNA. 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 water bath is exposed to ultrasound, allowing all the samples to be efficiently sonicated in parallel (Figure 1).

Use of Bioruptor® by pregnant women

Exposure to 30-60 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 30-60 kHz wave lengths for a long period of time.

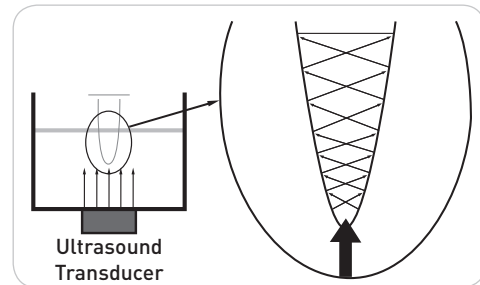


Fig 1. Propagation in 0.65 ml tubes

Bioruptor® Technical Specifications

Bioruptor® NGS	
Power Supply	100-230V, 50/60Hz, 2.1A (EU) / 4.2A (US)
Ultrasonic wave frequency	30-60 kHz
Ultrasonic wave output power	25-210 W
Transformer 220/100V dimensions	Internal
Control unit dimensions	350(W) x 260(D) x 165(H) mm
Sonication unit dimensions (water bath)	175(W) x 160(D) x 280(H) mm
Soundproof box dimensions	350(W) x 350(D) x 500(H) mm
Water bath volume	750 ml
Timer	Digital
Possibility to control water flow via connector kit for water cooler	Yes
Tube holder	Available for 0.65 ml tubes
Total weight	30 kg
Number of samples to be processed simultaneously	0.65 ml tubes – 12

Getting to Know Your Bioruptor® NGS System

Bioruptor® Components Overview



Control Unit



Water bath



Motorized lid



Soundproof box



Power Cable (EU)



Power Cable (US)



Control Unit Cable



Tube holder

Bioruptor® Water Cooler



To learn more, please visit www.diagenode.com

Connector kit for Water Cooler



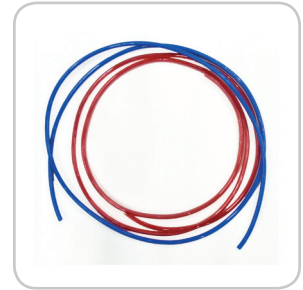
**Solenoid valve with
connector cable**



**Plastic connector for
motorized lid**



Male nozzle connector



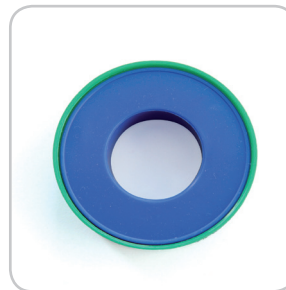
Tubing



Open-end wrench N° 14



Open-end wrench N°17



Plumbing Teflon



Spare Tubing

To learn more, please visit www.diagenode.com

Water bath

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

Handling

The water bath must remain upright at all times, especially when moved. Tilting the water 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 level and quality

The level of the water has been optimized and should always reach the red line (sticker on the wall of the tank). Distilled water should be used to fill the tank. Replacement stickers can be obtained from Diagenode.

Water temperature

The water in the water bath must be kept at **4°C**. Ultrasonic waves produced by the Bioruptor® generate heat. Drop off in sonication efficiency will occur above 10°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 Bioruptor® Water Cooler (Cat. No. BioAcc-Cool) has to be used in combination with the Connector kit for Water Cooler (Bioruptor® Plus & NGS) (Cat. No. VB-100-0001) to guarantee the automatic temperature control of the water bath during the entire sonication process (Figure 2). The cooling system features two pumps and produces a regular water flow to maintain a constant water level in the tank. The regulating valve (Connector kit for Water Cooler) ensures that water will only be replaced during the off cycle to avoid any interference between water flow and the sonication process.

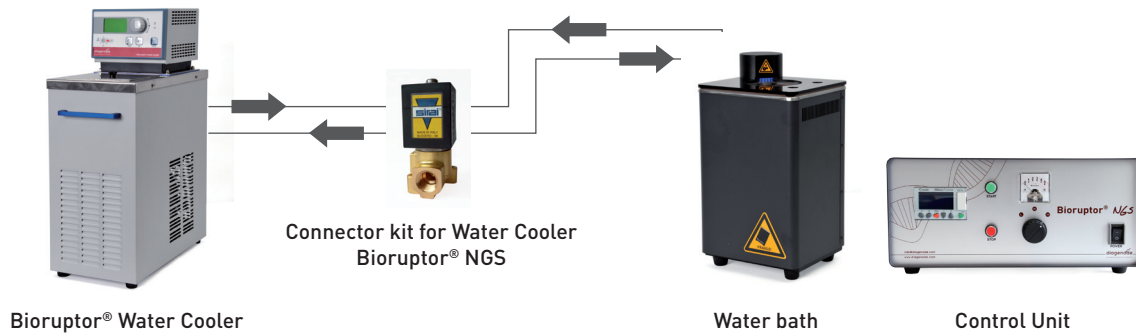


Fig 2. Setup of the Bioruptor® NGS System including Bioruptor® Water Cooler and Connector kit for Water cooler.

Note: You may permanently install the Bioruptor® in a cold room, though this is not sufficient to avoid the temperature increase during sonication.

Motorized Lid

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



Metallic Soundproof Box

This metallic soundproof box absorbs very efficiently the noise generated by the ultrasonic waterbath.

Tube Holders

The 0.5/0.65 ml tube holder allows for simultaneous sonication of up to 12 DNA samples. The optimal volume for DNA shearing is 100 µl and Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat. No. WA-005-0500) are recommended for processing your samples.



Equipment Installation

The following pages contain information on installing your particular Bioruptor® NGS 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 (beyond power connection) 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

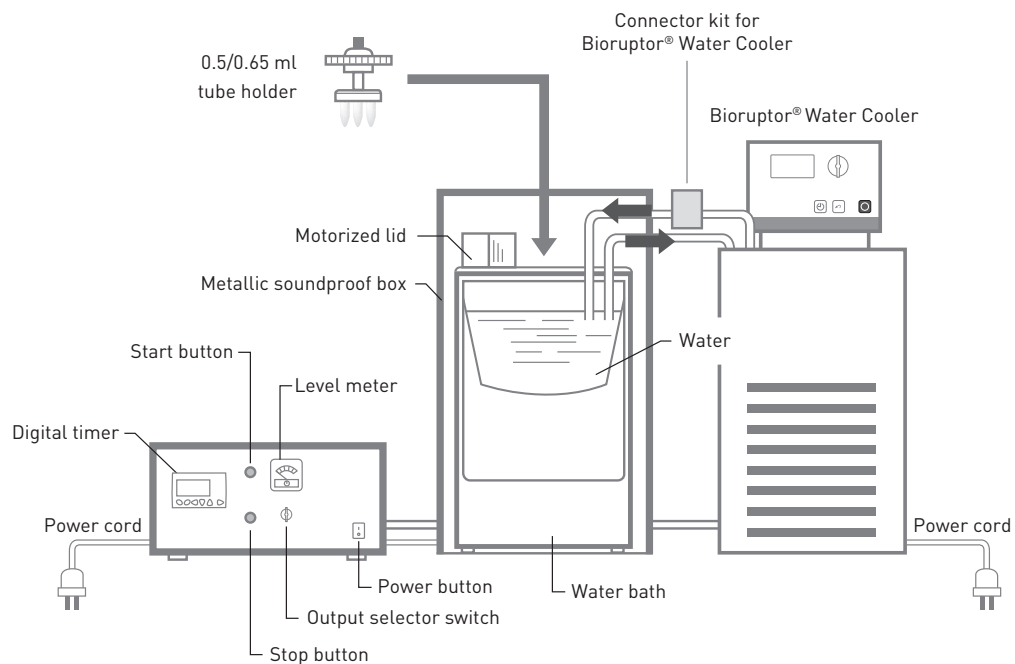


Fig 4. Schematic installation overview of the Bioruptor® NGS System in combination with the Bioruptor® Water Cooler and the Connector kit.

Installing the Bioruptor® NGS System with Water Cooler

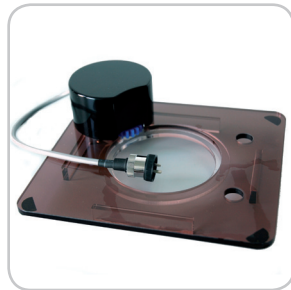
1. Open the boxes and unpack all components.



2. Place water bath in front of the soundproof box.
3. Remove the rubber cap (upper left hole marked machine cable) from the back of the soundproof box and feed the control unit cable through the hole.



4. Plug the control unit cable into the water bath. Make sure the red arrow on the plug is pointing up.



5. Place the 2 black plastic connectors on the motorized lid. Both connectors can be placed into one or the other hole of the motorized lid.

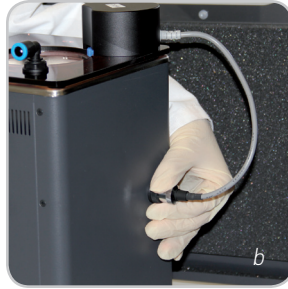
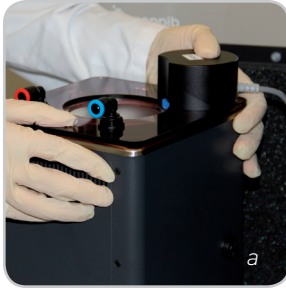
Screw the plastic nut over the motorized lid. The short red and blue pieces of tubing have to be inserted in the plastic connectors.



IMPORTANT NOTE:

If you need to cut a part of the tubing, always use the provided Cutting Device. Never use scissors because pieces of tubing with bad cuts generate leaks at junctions. Only properly cut tubes can be inserted in the connectors.

The length determines the water level in the sonication bath. After installation, if the water level is not correct, use the spare tubing and cut it at the right length (The suction pipe (red pipe) must be set at the same level as the water level sticker is).



6. Place the motorized lid (a) on the top of the water bath and connect it (b).

Bioruptor® Water Cooler & Connector kit

Location Requirements: The Water Cooler must be located underneath the Bioruptor® water bath.



Male nozzle connector

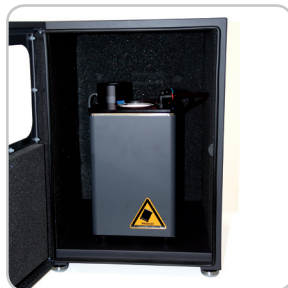


Solenoid valve

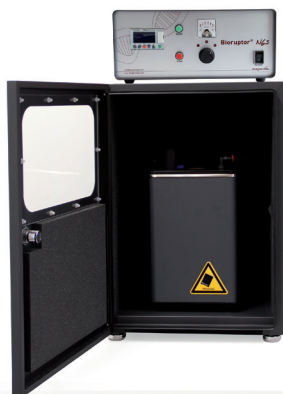
7. Unpack the Water Cooler and remove the black protection rubber which connects inlet and outlet.
8. Place the solenoid valve and the male nozzle connector on the outlet and inlet as shown in the picture. Use an open-end wrench N° 14 to install the male nozzle connector and N°17 to install the solenoid valve. Seal each thread by adding Plumbing Teflon (**Note:** Red connectors and red tubing will ensure the waterflow from the water bath to the Water Cooler. Blue connector and blue tubing will ensure the waterflow from the Water Cooler to the water bath).



9. Connect the Bioruptor® water bath to the Water Cooler with the red and blue tubes by inserting them in the appropriate connectors. (**Optional:** Cut the length you need for the output and input flow. Make sure there is enough slack.) The red and blue tubes must go through the soundproof box holes before being connected to the motorized lid (see picture).



10. Place the water bath into the soundproof box.



11. Place the control unit on the top of the soundproof box.



12. Plug the control unit cable into the control unit. Make sure the red arrow on the plug is pointing up.

13. Plug the power cable into the control unit.

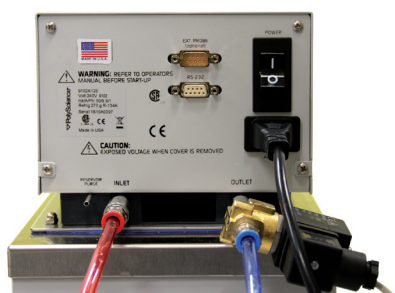


14. Plug the solenoid valve cable into the control unit.

15. Plug the power cable into the outlet and switch on the power switch on the back of the control unit.



16. Fill the water bath up to the red fill line with distilled water.



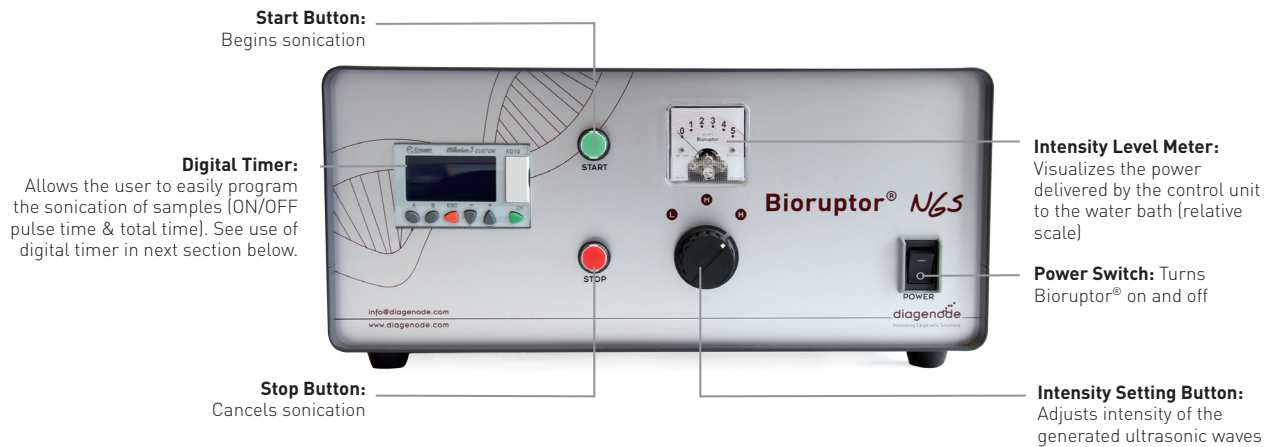
17. Plug the power cable into the back side of the Bioruptor® Water Cooler. Fill the tank of the circulator with 4.5 litres of water. Distilled water should be used in order to minimize the proliferation of bacteria.

18. Press first the power button on the back side of the Bioruptor® Water Cooler. Then press power button on the front side of the device. Temperature can be set by pushing and turning the knob on the first side of the device.

Note: See Bioruptor® Water Cooler manual for additional information.

Now you are ready to start !

Controlling the Sonication



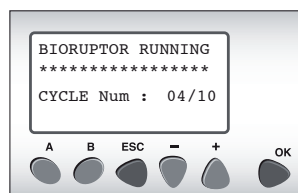
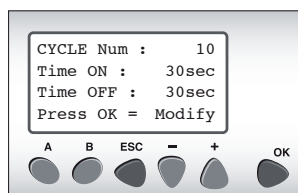
Switch on power switch (front side of control unit).

CYCLE NUMBER, TIME ON, 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.

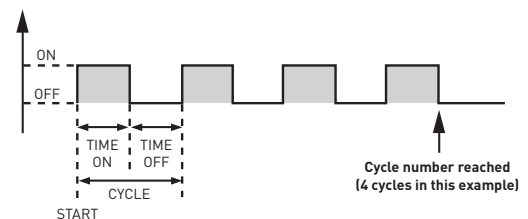
IMPORTANT NOTE: Minimum time of the off cycle: 30 seconds.

Digital Timer

After the introductory message, the control screen shows the main sonication parameters in the first two lines (CYCLE Number, Time) 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.

Tube holders & tubes



For DNA shearing we highly recommend to use the tube holder for 0.5/0.65 ml tubes (Cat. No. UCD-pack 0.5) and the corresponding Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat. No. WA-005-0500).

To use the tube holder, remove the lower part by turning counterclockwise. Then place microtubes in 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.

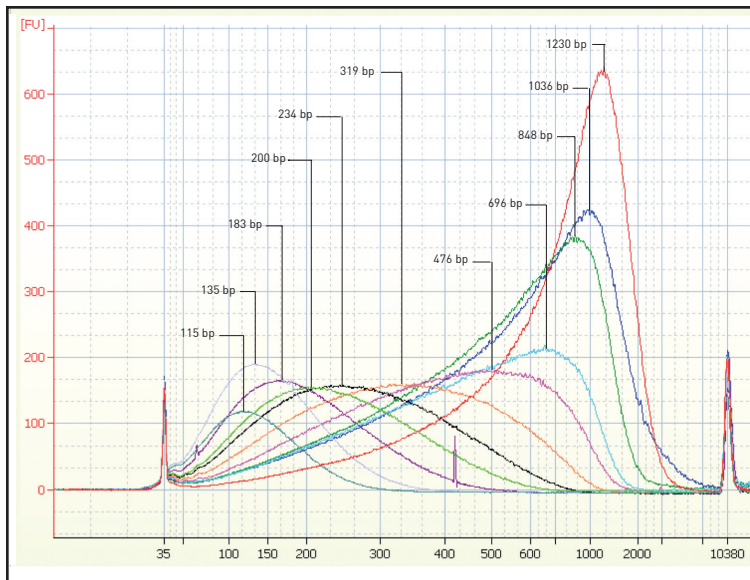


0.5/0.65 ml tube holder
(Cat. No. UCD-pack 0.5)



Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing
(Cat. No. WA-005-0500)

Standard protocols for DNA shearing



Programmable DNA size distributions, excellent reproducibility, and high dsDNA yields with Bioruptor® NGS

Figure shows different DNA size distributions of sheared genomic DNA produced by varying the duration of sonication using power setting high (H). The different curves depict a specific Bioruptor® NGS run, optimized to produce specific mean sizes and size ranges for Next-Generation sequencing. All samples were analyzed on Bioanalyzer 2100 using DNA High Sensitivity chip.

Operating conditions

Sample volume: 100 µl

Tubes: Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat. No. WA-005-0500)

Tube holder: 0.5/0.65 ml tube holder (Cat. No. UCD-pack 0.5) for 12 x 0.65 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 minutes prior to sonication.

Temperature: 4°C – Bioruptor® Water Cooler (Cat. No. BioAcc-Cool) & Single cycle valve for Water Cooler (Cat. No. VB-100-0001)

Power setting: H position (High)

Sonication cycle & total 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 condition (On/Off cycle time)	Cycle number
150 bp	30"/30"	30
200 bp	30"/30"	13
400 bp*	15"/90"	7 - 8
1000 bp*	5"/90"	7 - 8

* For longer fragments (400 up to 1000 bp), a short centrifugation step after half of the cycle numbers can significantly improve the results.

Protocols for other size ranges (incl. longer fragments up to 1300 bp) are available on request.

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® NGS 0.65 ml Microtubes for DNA Shearing [Cat No. WA-005-0500]. 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® NGS 0.65 ml Microtubes for DNA Shearing [Cat No. WA-005-0500] is 100 µl. When using lower volumes (e.g. ≤ 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 DNA concentration ranging between 1 and 20 ng/µl (10 ng/µl recommended). Using larger concentration (e.g. 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 minutes before sonication has also been shown to improve reproducibility.
- **DNA quality:** 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 (e.g. 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 (e.g. Agilent Bioanalyzer) or alter sonication efficiency.

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 (e.g. Proteinase K digested, double phenol/chloroform 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 cold water in the water bath. During sonication, especially when doing long sonication runs, the temperature must also be controlled. This is obtained by the automatic temperature control.

Note: The permanent installation of the Bioruptor® in a cold room is possible, although not sufficient to avoid the temperature increase due to sonication.

- **Automatic temperature control:** A recirculating water cooler is used to guarantee the automatic temperature control of the water bath during the whole sonication process. This water cooler (Cat No. BioAcc-cool) produces a regular water flow with a constant water level in the tank. An additional regulating valve (Single cycle valve for Water Cooler, Cat. No. VB-100-0001) ensures that water will only be replaced during the off cycle to avoid any interference the between water flow and the sonication process.
- **Sonication time:** Minor adjustments in cycle number may be made to optimize results for various sample types and concentrations. Cycle number listed above 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.
- **Water bath:** The sonication water 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. **Water bath maintenance:** The water bath metal surface is fragile and requires a careful maintenance. Use only soft sponge 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 (e.g. Agilent Bioanalyzer) or alter sonication efficiency.

Related Products

Diagenode develops and sells premium products for Epigenetics research that provide industry-leading sensitivity and consistency.

Diagenode offers a number of kits for Chromatin Immunoprecipitation (ChIP) and DNA methylation assays like Methylated DNA immunoprecipitation (MeDIP), MethylCap (MBD) and Bisulfite conversion (MagBisulfite kit). The Bioruptor®, with its powerful yet gentle ultrasound technology, allows for consistent shearing, a narrow size range of sheared DNA or chromatin, and sample preservation, necessary for successful experiments.

Antibodies

Diagenode offers a large selection of optimized ChIP & ChIP-seq grade, as well as MeDIP & MeDIP-seq grade antibodies that we have developed and characterized in-house.

For a complete listing of Diagenode's antibodies, please visit www.diagenode.com for more information.

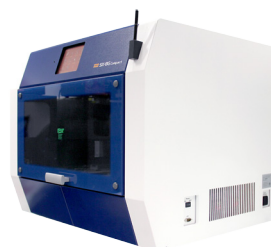


Chromatin Immunoprecipitation kits

Automated ChIP kits			
	Auto ChIP kit (1)	Auto Histone ChIP-seq kit (1)	Auto Transcription ChIP kit (1)
Features	All DNA-protein interaction, saving time, maximum reproducibility	Optimized for working with histone antibodies in ChIP-seq experiments, saving time, maximum reproducibility	Optimized for working with TF antibodies, saving time, maximum reproducibility
Optimized for	All DNA-protein interactions	Histones and histone modifications	Transcription factors and co-factors
Downstream applications	qPCR	qPCR, sequencing, arrays (2)	qPCR, sequencing, arrays (2)
Amount of cells/IP	1.000 - 1 million	1.000 - 10 million	1.000 - 10 million
Total Time of Assay	1 day	1 day	1 day
Handling time	30 min	30 min	30 min
Buffers and reagents	IP, DNA Isolation	IP	IP
Control antibodies	anti-IgG (rabbit)	anti-IgG (rabbit)	anti-IgG (rabbit)
DNA purification	DNA isolation buffer (DIB)	-	-
#rxns per kit	16 or 100	16 or 100	16 or 100
Cat. No.	AB-Auto01-A016 AB-Auto01-G016 AB-Auto01-A100 AB-Auto01-G100	AB-Auto02-A016 AB-Auto02-G016 AB-Auto02-A100 AB-Auto02-G100	AB-Auto03-A016 AB-Auto03-G016 AB-Auto03-A100 AB-Auto03-G100

(1) Validated on SX-8G IP-Star® and SX-8G IP-Star® Compact Automated Systems

(2) DNA purification has to be carried out with the IPure kit (Cat. No. AL-100-0100; not included in the kit).



Manual ChIP kits					
	LowCell# ChIP kit	HighCell# ChIP kit	Transcription ChIP kit	Histone ChIP kit	OneDay ChIP kit
Features	Magnetic bead-based protocol for all DNA-protein interaction, fast, increased DNA yield	Magnetic bead-based protocol Ideal to recover large amount of DNA (transcription factors, ChIP-on-chip) and to avoid biases due to amplification steps	Standard protocol with agarose beads optimized for transcription factors	Standard protocol with agarose beads optimized for histones and their modifications	Protocol using agarose beads quick and ready-to-use on large quantities of sheared chromatin, fast.
Optimized for	All DNA-protein interactions	All DNA-protein interactions	Transcription factors and co-factors	Histones and histone modifications	All DNA-protein interactions
Suitable for ChIP-seq and ChIP-on-chip	Yes (1)	Yes (1)	Yes	Yes	Yes
Amount of cells/IP	1.000 - 1 million	1 - 10 million	1 million	100.000	1.5 - 2 x 10e6
Total Time of Assay	1 day	1 day	3 days	3 days	1 day
Handling time	1.5 h	1.5 h	2h	2h	2h
Buffers and reagents for	Cell lysis, chromatin shearing, IP, DNA purification	Cell lysis, chromatin shearing, IP, DNA purification	Cell lysis, chromatin shearing, IP	Cell lysis, chromatin shearing, IP	IP, DNA purification
Control antibodies	anti-IgG (rabbit)	anti-IgG	anti H3 (K4me3)	anti-TBP or RNA Pol II (2)	anti-IgG (rabbit)
Control PCR primer pairs	human TSH2B / c-fos / myoglobin exon 2	human TSH2B / GAPDH promoter	BMX / c-fos / beta actin / myoglobin exon 2	GAPDH / 0,5 kb / idem -1 kb / c-fos / beta actin / myoglobin exon 2	-
DNA purification	DNA isolation buffer	DNA isolation buffer	Not included	Not included	DNA purifying slurry
#rxns per kit	16 or 48	16	18	18	60 / 180
Cat. No.	kch-maglow-A16 kch-maglow-G16 kch-maglow-A48 kch-maglow-G48	kch-mahigh-A16 kch-mahigh-G16	kch-redTBP-012	kch-orgHIS-012	kch-oneDIP-060 kch-oneDIP-180

(1) DNA purification has to be carried out with the IPure kit (Cat. No. AL-100-0100; not included in the kit).

(2) Please mention your choice of antibody on your purchase order.

DNA methylation kits

Automated DNA methylation kits			
	Auto MethylCap kit (1)	Auto MeDIP kit (1)	Auto hMeDIP kit (1)
Features	Allows to capture fractions of methylated DNA by CpG density. Includes control primer pairs for assessment of capture efficiency	Quality control using internal controls, improved handling (magnetic beads), high specificity (monoclonal 5-meC Ab), fast	Quality control using internal controls, improved handling (magnetic beads), high specificity (monoclonal 5-hmC Ab), fast
Downstream applications	qPCR, linear amplification and genome-wide analysis such as microarray and sequencing (2).	qPCR, linear amplification and genome-wide analysis such as microarray and sequencing (2).	qPCR, linear amplification and genome-wide analysis such as microarray and sequencing (2).
Amount of DNA/rxn	1 µg	1 µg	1 µg
Total Time of Assay	1 day	1 day	1 day
Handling time	30 min	30 min	30 min
Internal controls	-	Methylated and unmethylated BAC clones	Hydroxymethylated, methylated and unmethylated BAC clones
Control PCR primer pairs	TSH2B/GAPDH	Methylated DNA control unmethylated DNA control	Hydroxymethylated DNA control methylated DNA control unmethylated DNA control Sfi1 for genomic DNA
#rxns per kit	48	16 or 100	16
Cat. No.	AF-Auto01-0048	AF-Auto01-A016 AF-Auto01-G016 AF-Auto01-A100 AF-Auto01-G100	AF-Auto02-0016

(1) Validated on SX-8G IP-Star® and SX-8G IP-Star® Compact Automated Systems

(2) DNA purification has to be carried out with the IPure kit [Cat. No. AL-100-0100; not included in the kit].

Manual DNA methylation kits					
	MethylCap kit	MagMeDIP kit	MeDIP kit	hMeDIP kit	MagBisulfite kit
Features	Allows to capture fractions of methylated DNA by CpG density, magnetic beads permit fast and sensitive capture, includes control primer pairs for assessment of capture efficiency	Quality control using internal controls, improved handling (magnetic beads), high specificity (monoclonal 5-meC Ab), fast	Quality control using internal controls, high specificity (monoclonal 5-meC Ab), agarose beads, fast	Quality control using internal controls, improved handling (magnetic beads), high specificity (monoclonal 5-hmC Ab), fast	Gives precise information on methylation status of single cytosines. High conversion rate →99%. DNA purification based on magnetic beads and compatible with SX-8G IP-Star® Automated System.
Suitable for	qPCR, linear amplification and genome-wide analysis (microarray, sequencing). Depending on the DNA purification method selected	qPCR, linear amplification and genome-wide analysis (microarray, sequencing). Depending on the DNA purification method selected	qPCR, linear amplification and genome-wide analysis (microarray, sequencing). Depending on the DNA purification method selected	qPCR, linear amplification and genome-wide analysis (microarray, sequencing). Depending on the DNA purification method selected	qPCR, sequencing, microarray
Amount of DNA/rxn	1 µg	1 µg	1 µg	1 µg	1 ng - 1 µg
Total Time of Assay	1 day	2 or 3 days	2 or 3 days	2 or 3 days	3.5 hours
Handling time	2h	1.5 h	2h	1.5 h	2h
Internal controls	-	Methylated and unmethylated BAC clones	Methylated and unmethylated BAC clones	Hydroxymethylated, methylated and unmethylated BAC clones	-
Control PCR primer pairs	TSH2B/GAPDH	Methylated DNA control unmethylated DNA control TSH2B GAPDH	Methylated DNA control unmethylated DNA control TSH2B GAPDH	Hydroxymethylated DNA control methylated DNA control unmethylated DNA control Sfi1 for genomic DNA	Bisulfite-specific primer pair
#rxns per kit	48	10 or 48	10	16	24
Cat. No.	AF-100-0048	mc-magme-A10 mc-magme-048	mc-green-003	AF-104-0016 AF-110-0016 AF-111-0016	AF-106-0024

Ordering information

Description	Cat. No.
Bioruptor®	
Bioruptor® NGS with 0.5/0.65 ml tube holder	UCD-600 TS
Tube Holders	
0.5/0.65 ml tube holder for Bioruptor® Standard, Bioruptor® Plus & Bioruptor® NGS	UCD-pack 0.5
Cooling System	
Bioruptor® Water Cooler including standard connectors for Bioruptor®	BioAcc-cool
Connector kit for Water Cooler (Bioruptor® Plus & Bioruptor® NGS)	VB-100-0001
Tubes	
Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing	WA-005-0500

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