diagenode

Innovating Epigenetic Solutions

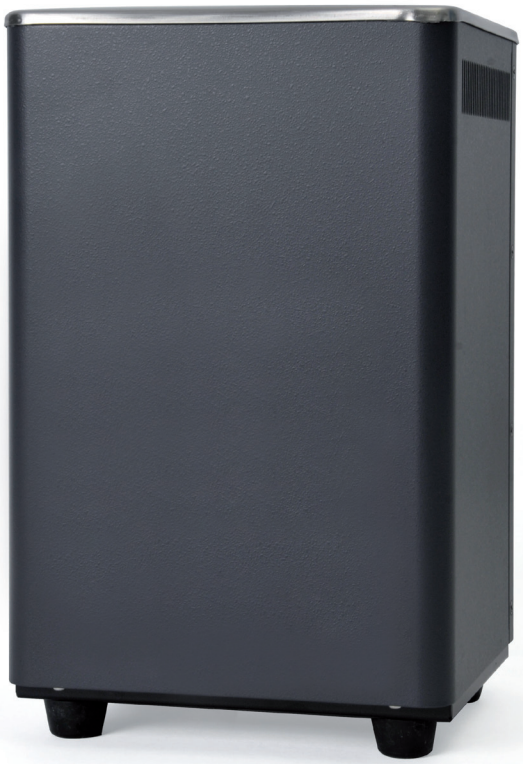
Bioruptor®
Sonication System

USER MANUAL

Version 1.1

Bioruptor® Standard

Cat. No. UCD-200



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 level (water bath)

- 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 temperature (water bath)

- Optimal water temperature for sonication is 4°C. Sample temperature should not exceed 10°C.
- Methods to maintain the temperature.
  - Ice - add small amounts of crushed ice (no more than 0.5 cm) to the bath every 10 minutes.
  - Bioruptor® Water Cooler (Cat. No. BioAcc-Cool) - the flow rate of the circulating water cooler cannot exceed 500 ml per minute for optimal sonication.

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® Standard

- DNA shearing: Bioruptor® 0.5 ml Microtubes for DNA Shearing (Cat. No. WA-004-0500).
- Chromatin Shearing: 1.5 ml TPX microtubes (Cat. No. M-50050 or M-50001) and 15 ml TPX tubes (Cat. No. M-UN-15).

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 (0.5/0.65 ml tube holder - Cat. No. UCD-pack 0.5 or 1.5 ml tube holder - Cat. No. UCD-pack1.5). 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.

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® Standard 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 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.

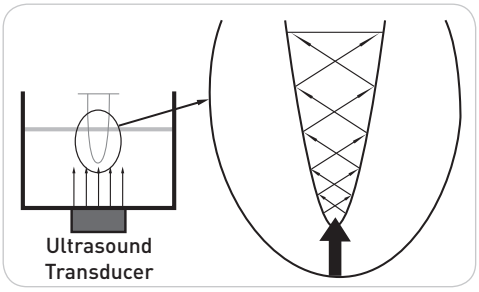


Fig 1. Propagation in 0.5 ml tubes and 1.5 ml tubes

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.

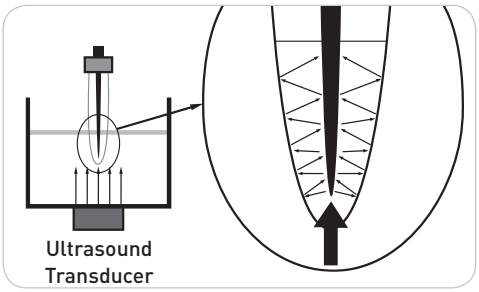


Fig 2. Propagation in 15 ml & 50 ml tubes

With the Bioruptor®, the entire volume of water present in the waterbath 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 waterbath 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	115 V/ 4.2A (US) // 230V/2.1A (EU), 50/60Hz
Control unit dimensions	150(W) x 285(D) x 195(H) mm
Sonication unit dimensions (water bath)	175(W) x 160(D) x 265(H) mm
Soundproof box dimensions	350(W) x 350(D) x 500(H) mm
Water bath volume	750 ml
Timer	Analog
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® Standard System

Bioruptor® Components Overview

Control Unit

Water bath

Motorized lid

Soundproof Box

Control Unit Cable

Tube holder example

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 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.

Manual temperature control

- A "precooling" of the Bioruptor®'s tank 15 minutes before starting the first round of sonication is advised. This prevents the water from heating too quickly due to thermal inertia (i.e. when the tank and the ultrasound generating elements are stored at room temperature). To precool, simply add crushed ice and then fill with cold water up to the indicated level (red line on the water level sticker).
- Every 10 minutes replace crushed ice. The ice floating in the water should not exceed 0.5 cm and the total water level (water & ice) should be exactly at the indicated water level.

- Fill entirely a 250 ml beaker with crushed ice
- Pour ice carefully into your sonication water bath (which is already filled with water to the red fill line)
- Remove approximately 130 ml of water (without ice)
- Carefully adjust water level to the indicated mark (red line) by removing or adding water using a pipette

Automatic temperature control

The Bioruptor® Water Cooler (Cat. No. BioAcc-Cool) guarantees the automatic temperature control of the water bath during the entire sonication process (Figure 3). The cooling system features two pumps and produces a regular water flow to maintain a constant water level in the tank.

Fig 3. Setup of the Bioruptor® Standard System in combination with the Bioruptor® 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. The cold room would only eliminate the need for the "precooling" step described above.

Motorized Lid

The motorized lid, along with the blue 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 more than 30 dBA generated by the ultrasonic waterbath (Figure 4).

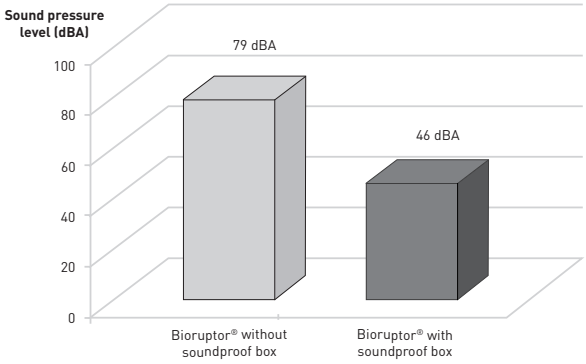
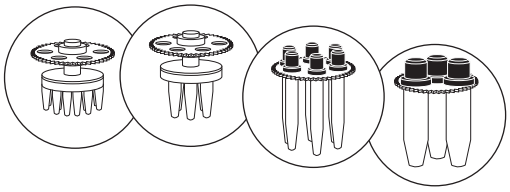


Fig 4. Sound pressure of the Bioruptor® without and with metallic soundproof box has been measured in a soundproof room.

Tube Holders

Several sizes of tubes can be used with the Bioruptor® Standard. 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 metallic bar has to be used to reflect sound waves to produce a better resonance 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® Standard 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

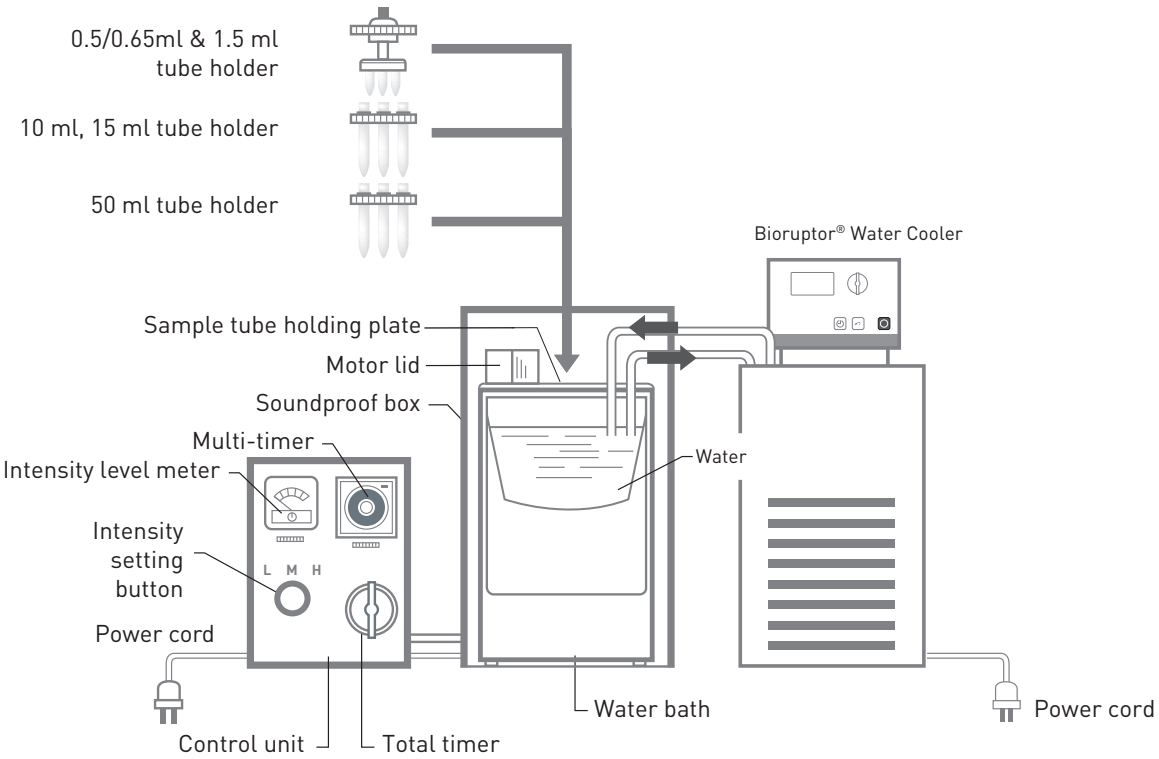


Fig 5. Schematic installation overview of the Bioruptor® Standard System in combination with the Bioruptor® Water Cooler.

Installing the Bioruptor® Standard System

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.

5. Place the motor lid on the top of the water bath and connect it.

6. Place the water bath into the soundproof box.

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

8. Plug the control unit cable into the control unit.

9. Plug power cable into the control unit.

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

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

Now you are ready to start !

Controlling the Sonication

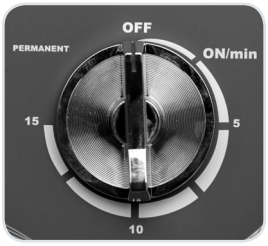
CYCLE NUMBER, TIME ON, TIME OFF and sonication **INTENSITY** are the key parameters controlling the sonication.

Multi-timer: The control unit allows the automatic production of ON / OFF cycles to preserve the samples from rapid heating due to the ultrasound energy. This is achieved by the Multi-timer located on the upper right part of the control unit.

30 sec « ON »/ 30 sec « OFF » cycle

30 sec « ON »/ 90 sec « OFF » cycle

15 sec « ON »/ 180 sec « OFF » cycle



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 default unit is the minute. The time units (min or sec) can be modified by using a screw driver on the screws directly above the « ON » and « OFF ». Three examples of « ON » and « OFF » cycles set on the multi-timer are shown above.

Total (analog) timer: The Bioruptor® Standard is activated by the clockwise rotation of this dial. It has 4 positions: 5 min - 10 min - 15 min - permanent (for any total sonication time higher than 15 min). The maximum time is 15 minutes. The time set will equal the sum of the "ON" and "OFF"cycles. Alternatively, turning the timer knob counterclockwise will set the Bioruptor® Standard on a permanent position (always cycles "ON" and "OFF"). To stop this process, manually set the timer knob on to the vertical position.

Intensity setting button: 3 power settings are available for the sonication: **L** (Low), **M** (Medium) and **H** (High).

Intensity level meter: allows visualization of the power delivered by the Control Unit to the water bath (relative scale).

Tube holders & tubes

Tube holders - Available for 0.5/0.65 ml, 1.5 ml, 10 ml, 15 ml, and 50 ml tubes

To use the 0.5/0.65 ml & 1.5 ml tube holder, remove the lower part of the holder by turning counterclockwise. Then place tubes into the unit. Attach the lower part to the upper part of the tube holder. To guarantee homogeneity of sonication, the tube holders should always be completely filled with tubes. To ensure reproducibility, always use the same brand of tubes. Bioruptor® 0.5 ml Microtubes for DNA Shearing (Cat. No. WA-004-0500) are recommended for DNA shearing.

- 1.5 ml TPX microtubes (Cat. No. M-50050/M-50001) provide better ultrasound transfer rates and more efficient sonication. These tubes should be used for chromatin shearing, not DNA shearing. Any 0.5 ml or 1.5ml tubes can be used although shearing efficiency might be lower. The 2 ml polypropylene tubes (thin-walled) should not be used with the Bioruptor®.
- The complete tube holder (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 for more information).

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

1.5 ml tube holder
(Cat. No. UCD-pack 1.5)

10 ml tube holder
(Cat. No. UCD-pack 10)

15 ml tube holder (Cat. No. UCD-pack 15)

The tube holders for 15 ml tubes are available for Falcon & Corning tubes. If you use another brand of tubes, use the one which fits in the holder the best. 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. UCD-pack 50)

The tube holders for 50 ml tubes are available for Falcon tubes (blue) and for Corning tubes (orange). If you use another brand of tubes, use the one which fits in the holder the best.

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 for the different tube types (hard plastic is more efficient).

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 10ml, 15ml, 50ml 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. UCD-pack 0.5) and the corresponding Bioruptor® 0.5 ml Microtubes for DNA Shearing (Cat. No. WA-004-0500).

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

Bioruptor® 0.5 ml Microtubes for DNA Shearing
(Cat. No. WA-004-0500)

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. WA-004-0500)

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

Temperature: Maintain at 4°C by using ice chilled water and small amounts of crushed ice (no more than 0,5 cm) or use the Bioruptor® Water Cooler (Cat. No. BioAcc-Cool).

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.)	Total sonication time in min (On + Off times)
1250 bp	15/90	3.5 min
950 bp	15/90	7 min*
750 bp	30/90	6 min*
550 bp	30/90	10 min
400 bp	30/90	12 min
350 bp	30/90	16 min
300 bp	30/90	20 min
250 bp	30/90	30 min
200 bp	30/90	60 min
150 bp	30/30	70 min

*recommended to use a lab timer to set time precisely

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. WA-004-0500). 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® 0.5 ml Microtubes for DNA Shearing (Cat No. WA-004-0500) 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 minutes 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 (eg. 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.

  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 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.

- **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.

- **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 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.
- SDS is a key component of sonication buffer for chromatin shearing. Include 0.7-1% of SDS in your 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 minutes 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. M-50050 or M-50001).

9. Sonicate samples with Bioruptor® Standard with refrigerated water bath (or crashed ice water 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 14000 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, kch-redmod-100).
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. M-50050 or M-5001).

6. Sonicate samples with Bioruptor® Standard with refrigerated water bath (or crashed ice water 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 14000 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. M-50050) or 10 ml tubes (Cat. No. AS-100) and the corresponding tube holders (Cat. No. UCD-pack 1.5 and UCD-pack10). 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. UCD-pack 1.5) or 10 ml tubes holder (Cat. No. UCD-pack 10) 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 Bioruptor® Water Cooler (Cat. No. BioAcc-Cool) or by using crushed ice

Power setting: H position (High)

Sonication cycle: 30 sec ON, 30 sec OFF

Total sonication time: 10 min for UCD200/300
15 min for Bioruptor XL

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 1000 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 (UCD200/300) 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 minutes. Two types of tubes were tested: Diagenode's 1.5 ml TPX tubes (M-50001) and Diagenode's 10 ml tubes (AS-100). 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 minutes 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 minutes of sonication.

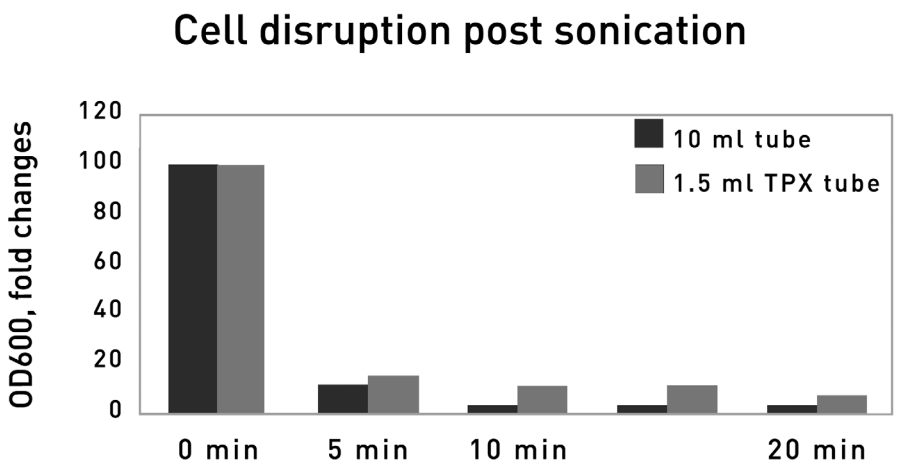
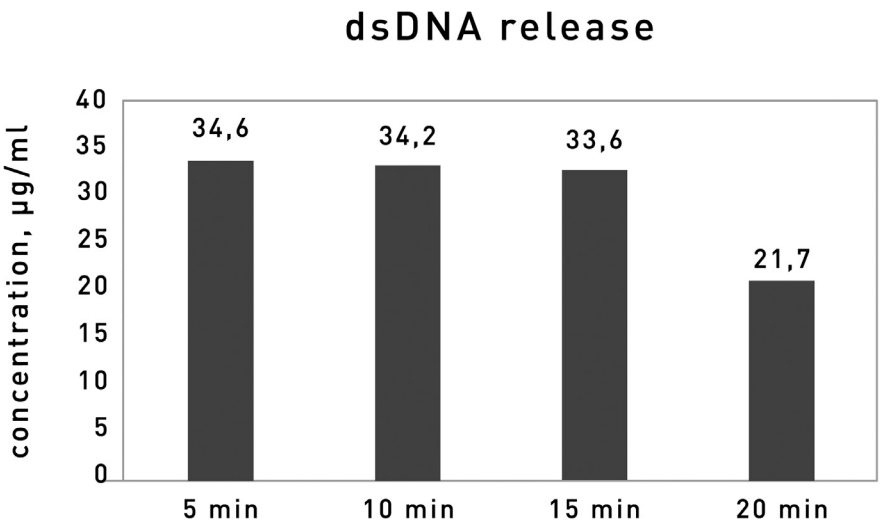


Figure 1: Effect of sonication on cell disruption
The number of intact cells after sonication was determined by measuring optical density at 600 nm. Optical density of the cell culture before sonication (0 min) is arbitrarily set to 100%.

Sheared DNA is released during bacterial sonication

The disruption of bacterial cells by sonication releases DNA with maximum recovery after only 5 minutes of treatment (Fig.2, A). The released DNA is fragmented with fragment size dependent on sonication time (Fig.2, B).

A.



B.

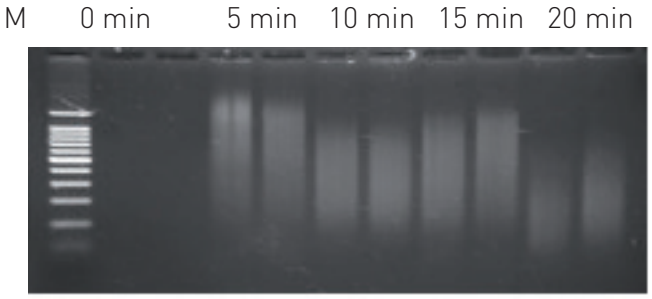


Figure 2 : Effect of sonication on DNA release

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 minutes (with a time course when needed)	It is possible to fix for as little as 5 minutes (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 minutes. If 30', interrupt sonication after every 10 minutes 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 waterbath 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.

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 antbodies 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 anbosies, 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).

SX-8G IP-Star® Compact Automated System

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 biais 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

diagenode

Innovating Epigenetic Solutions

Ordering information

Description	Cat. No.
Bioruptor® Models	
Bioruptor® Standard	UCD-200 TM (1.5 ml) UCD-200 TO (1.5 ml & 15 ml) UCD-200 TS (0.5/0.65 ml)
Bioruptor® Plus	UCD-300 TM (1.5 ml) UCD-300 TO (1.5 ml & 15 ml) UCD-300 TS (0.5/0.65 ml)
Bioruptor® Twin	UCD-400 TM (1.5 ml) UCD-400 TO (1.5 ml & 15 ml)
Bioruptor® XL	UCD-500 TM (1.5 ml) UCD-500 TO (1.5 ml & 15 ml)
Bioruptor® NGS	UCD-600 TS (0.5/0.65 ml)
Cooling System	
Bioruptor® Water Cooler including continuous valve for Bioruptor®	BioAcc-cool
Peristaltic pump - including standard connectors	TWI-pump
Consumables	
1.5 ml TPX Microtubes	M-50001
1.5 ml TPX Microtubes	M-50050
15 ml TPX Microtubes	M-UN-15
Bioruptor® 0.5 ml Microtubes for DNA Shearing	WA-004-0500
Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing	WA-005-0500

Description	Cat. No.
Connector Kits	
Single Cycle Valve for Bioruptor® Water Cooler (Bioruptor® Plus & NGS)	VB-101-0001
Dual Cycle Valve for Bioruptor® Water Cooler (Bioruptor® Twin)	VB-100-0001
Tube Holders	
0.5/0.65 ml tube holder for Bioruptor® Standard, Bioruptor® Plus & Bioruptor® NGS	UCD-pack 0.5
1.5 ml tube holder for Bioruptor® Standard & Bioruptor® Plus	UCD-pack 1.5
10 ml tube holder for Bioruptor® Standard & Bioruptor® Plus	UCD-pack 10
15 ml tube holder for Bioruptor® Standard & Bioruptor® Plus	UCD-pack 15
50 ml tube holder for Bioruptor® Standard & Bioruptor® Plus	UCD-pack 50
0.5/0.65 ml tube holder for Bioruptor® Twin	TWI-pack 0.5
1.5 ml tube holder for Bioruptor® Twin	TWI-pack 1.5
10 ml tube holder for Bioruptor® Twin	TWI-pack 10
15 ml tube holder for Bioruptor® Twin	TWI-pack 15
50 ml tube holder for Bioruptor® Twin	TWI-pack 50
0.5/0.65 ml tube holder for Bioruptor® XL	XL-pack 0.5
1.5 ml tube holder for Bioruptor® XL	XL-pack 1.5
10 ml tube holder for Bioruptor® XL	XL-pack 10
15 ml tube holder for Bioruptor® XL	XL-pack 15
50 ml tube holder for Bioruptor® XL	XL-pack 50

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