Diagenode Bioruptorpico Ultrasonication System

STANDARD OPERATION PROTOCOL

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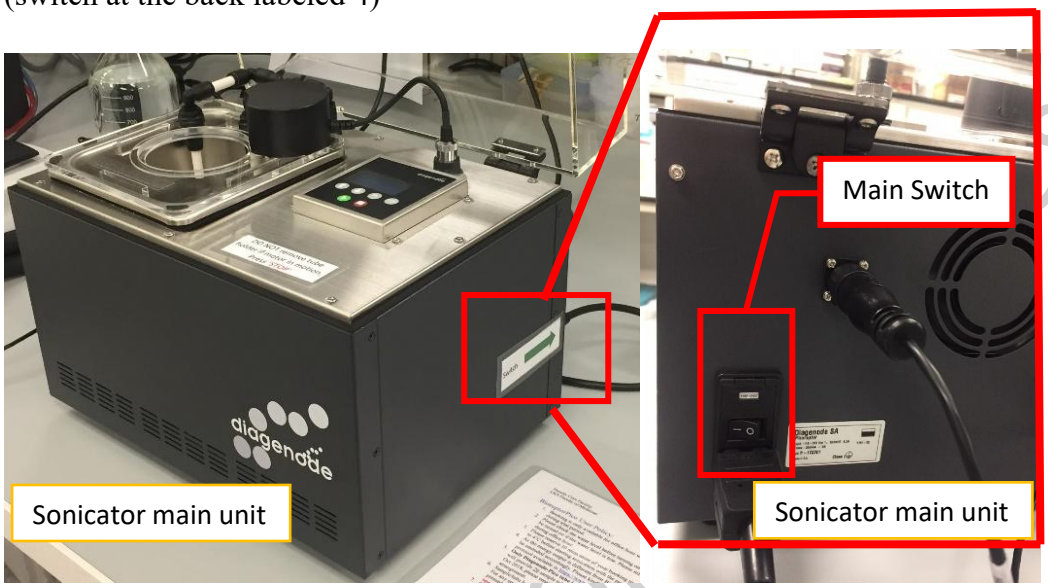
Standard Operating Protocol

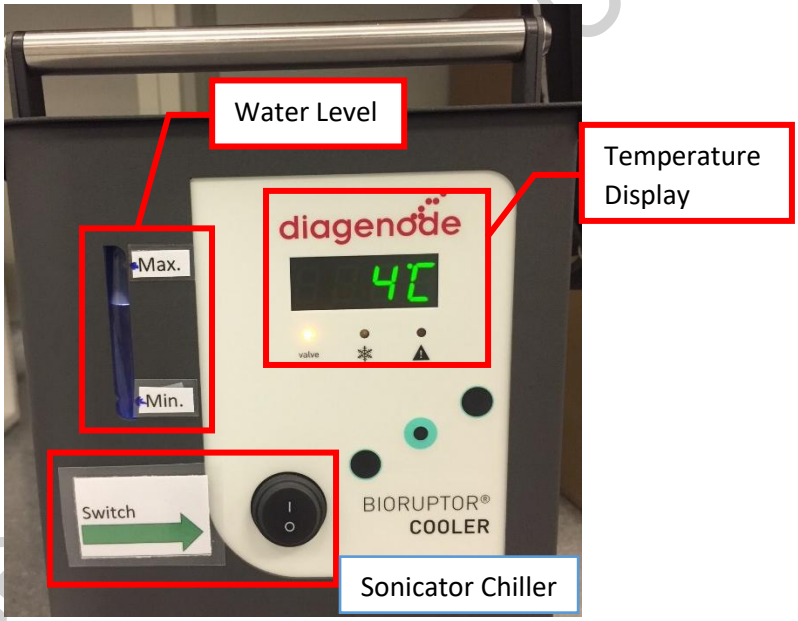
I. Important Note:

1. Check the water level of the sonication bath unit and the chiller before turning on the system. The system is not allowed to be turned on if the water level is low.
2. Turn on the chiller (Labeled 1 to 3) 20 mins before booking starts to cool down to 4˚C.
3. Pre-cool and spin all samples before the start of sonication.
4. Energy output of BioruptorPico is different from BioruptorPlus, your protocol should be amended accordingly. Please kindly optimize your experiment based on the protocol available on https://www.diagenode.com/en/protocols
5. **Only Diagenode-Pico tube is allowed for your experiment using this system**.
6. Sample/tube holder **MUST NOT** be removed from the motor when it is still running. Pressed '**Stop**' button before removing sample. **The user needs to cover the cost of replacement of the motor if the rotor is damaged.**
7. System is required to be **rested for 5 mins for every 10 cycles of operation, and for 10 mins for every 30 mins of operation** to maintain constant power output and to avoid overheating.
8. **For sonication program, ON time must ≤ OFF time,** otherwise the system will be overheated and broken. Please consult CPOS staff if you would like to set a new sonication program.
9. You are required to turn off the system unless with the next user's acknowledgement.

II. Initialization

1. Check Water level at sonication bath unit and chiller, fill up water as required (Distilled water only)
2. Turn on the Chiller (switches at the front labeled 1 to 3) and the Sonicator main unit (switch at the back labeled 4)





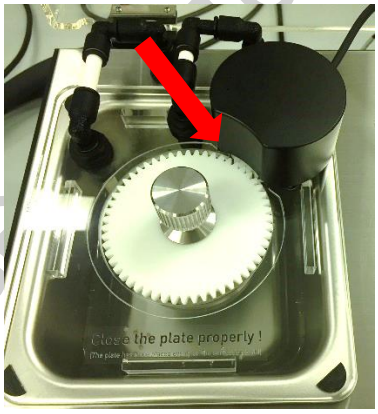
3. Chiller takes around 20 mins to cool down the system and water to 4˚C

4. Set up program according to Protocol available on https://www.diagenode.com/en/protocols And https://pybrevet.typeform.com/to/o8cQfM

    a. Press and hold 'OK' to reset the parameters
    b. Set Time On, Time Off and Cycle Number by using '▲' and '▼' to adjust parameters, then press 'OK' to confirm

    c. Time ON must always be **less than or equal to** Time OFF
    d. It's recommended to split your total cycle number into two rounds, centrifuge samples in between the two rounds for better sonication efficiency (e.g. Total cycle number=20; Set round 1 for 10 cycles, centrifuge your sample, start round 2 for another 10 cycles)

5. Sample should be loaded in Diagenode Pico tube. Vortex and centrifuge to remove any air bubbles, insert to opposite tube holder
6. Ensure all the tubes are tightly closed and placed **flat** on the tube holder well before screwing the top rotor. **Fill up all unused slots** with the same volume of deionized water as in your sample for consistent sonication.
7. Keep your samples on ice before sonication.
8. Insert the tube holder carefully without damaging the gear.



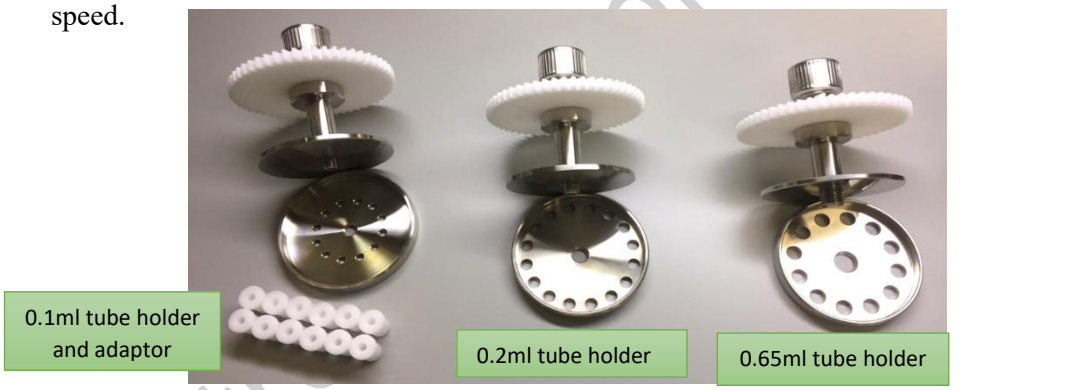
9. Press '▶' and close the lid to start the run.
10. If there's emergency to remove the tube holder during the program, **DO NOT** remove the tube holder when the gear is still in motion, press STOP '■', then remove the tube holder. Please note that the current running cycles setting will be discarded, please check the cycle number before pressing STOP.

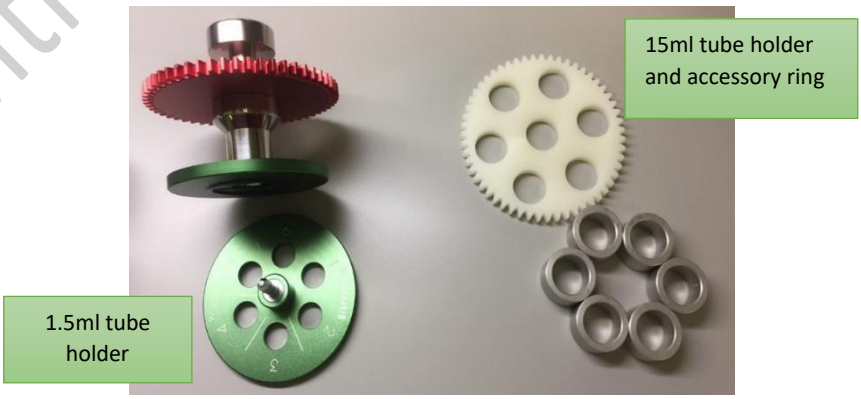
11. The motor will stop running when the run is completed, open the lid and remove the tube holder carefully
12. Rest the system for 5 mins for every 10 cycles of operation, and 10 mins for every 30 mins of operation.
13. Repeat for other samples or **turn off** the system (switches from 4 to 1) unless with the acknowledgement from the next user. Also turn off the switches on wall socket.
14. Dry the tube holder and balancing tubes and return them to the corresponding tray gently.
15. Sign on the **log sheet** before leaving.

III. Tubes and Adaptors

Cat. No.	DNA/ Chromatin shearing	Tube size	No. of sample	Min. volume	Max. volume
C30010015	DNA/ Chromatin	0.1 ml	12	5 or 10 µl	50 µl
C30010013	DNA/ Chromatin	0.2 ml	16	20 µl	100 µl
C30010011	DNA	0.65 ml	12	100 µl	100 µl
C30010016	Chromatin	1.5 ml*	6	100 µl	300 µl
C30010017/ C30010021 (with Protein extraction beads)	Chromatin	15 ml*	6	500 µl	2 ml

*Do not centrifuge 1.5ml and 15 ml Diagenode tubes over 5000 rpm. The tube will break upon high speed.





- Diagenode Plus tubes and adaptors are not suitable for Diagenode Pico system, only Diagenode Pico tube is acceptable for Pico application.

Tube	Sample volume	DNA concentration	Target size	Cycle condition (On/Off cycle time)	Cycle number (short centrifugation step)
0.1 mL	10 µL	10 ng/µL	200 bp	30”/30”	20
			250 bp	30”/30”	15 (5, 10)
			300 bp	30”/30”	13 (4, 8)
			550 - 600 bp	15”/90”	7 (3)
			750 - 800 bp	15”/90”	6 (3)
0.1 mL	50 µL	10 ng/µL	150 bp	30’’/30’’	30
			200 bp	30’’/30’’	10 (6)
			300 bp	30’’/30’’	7 (3)
			350 - 400 bp	15’’/90’’	7-8 (3)
			1000 bp	5’’/90’’	6 (3)
0.65 mL	100 µL	1-20 ng/µL	150 bp	30’’/30’’	30
			200 bp	30’’/30’’	13
			300 bp	30’’/90’’	6 (3)
			400 bp	15’’/90’’	7-8 (3)
			1000 bp	5’’/90’’	7-8 (3)

Reference setting for DNA shearing.

As the power output is different from BioruptorPlus, your protocol should be amended accordingly. Please reference to https://www.diagenode.com/en/protocols and https://pybrevet.typeform.com/to/o8cQfM for details.