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Sample Preparation for Automated Extraction in Periphyton Metabolomic Analysis V.2

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Lin Zi[1], Mélissa EON[1], Nicolas Creusot[1]
[1]INRAE EABX

MetaboHUB-Bordeaux

Lin Zi
INRAE

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We use this protocol and it's working

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Abstract

This protocol details the sample preparation process using micronic tubes for automated robotic extraction in periphyton metabolomics. The procedure utilizes periphyton previously established on 9-mm glass discs and exposed to chemical stressors in a 48-well plate format to evaluate ecotoxicological risk.

Guidelines

- Since the procedure of measuring dry biomass without caps, it’s better to do the measurement fast and close the lid of the micronic plate whenever it’s possible to avoid contamination of the samples.

- Try to limit freeze and de-freeze samples before the samples are dry.

Materials

micronic 96-well plate

micronic 1.1 mL tubes

micronic screw caps

metal bead

GENEVAC EZ2

Safety warnings

Safety: Wear a lab coat, gloves, and safety glasses; utilize the fume hood and follow chemical safety rules. Follow safety rules for using liquid nitrogen.
Waste: Recycle all waste according to quality document EABX_GEN_706.
Traceability: Optimize labeling and storage for all samples, reagents, and stock solutions.
Monitoring: Perform gravimetric monitoring for all stock solutions.
Documentation: Record the weight, volume, and chemical nature of all internal standards in the laboratory notebook.

Before start

Periphyton samples were colonized on 9-mm glass discs and subsequently exposed to chemicals within a 48-well plate format to evaluate chemical risk. The colonization procedure has been described in "Periphyton growing and sampling in situ or in mesocosm for metabolomic analysis.**protocols.io** https://protocols.io/view/periphyton-growing-and-sampling-in-situ-or-in-meso-dmxt47nn". The contamination procedure has been described in "Contamination procedure for chemical risk assessment in periphyton.**protocols.io** https://protocols.io/view/contamination-procedure-for-chemical-risk-assessme-hgxyb3xpx ".

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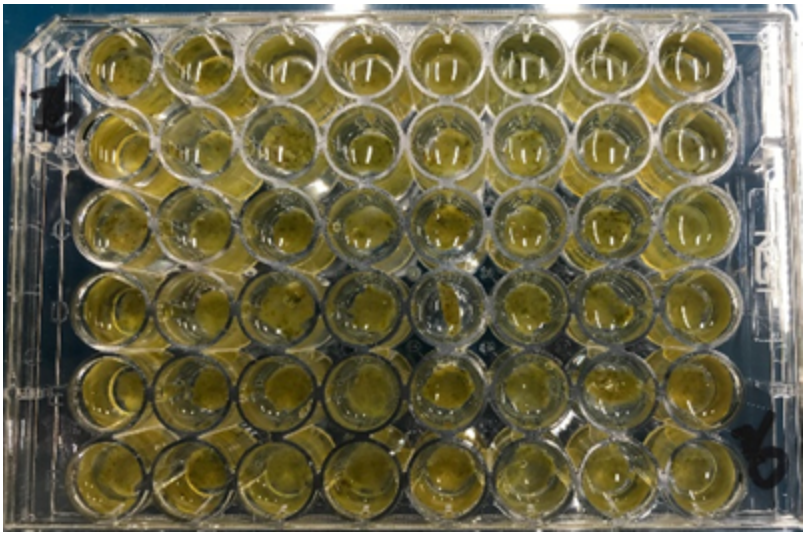


Periphyton grown on 9-mm glass discs in 48-well plate

Quenching

5m

1 After chemical exposure in the 48-well plate, discard 850 µL medium from each well, leaving approximately 150 µL inside the well to prevent periphyton desiccation. If plan to perform exometabolome analysis, transfer 850 µL medium from each well to 1.1 mL Micronic tube. To ensure sufficient space for capping, discard 150 µL of the transferred volume (leaving 700 µL medium).

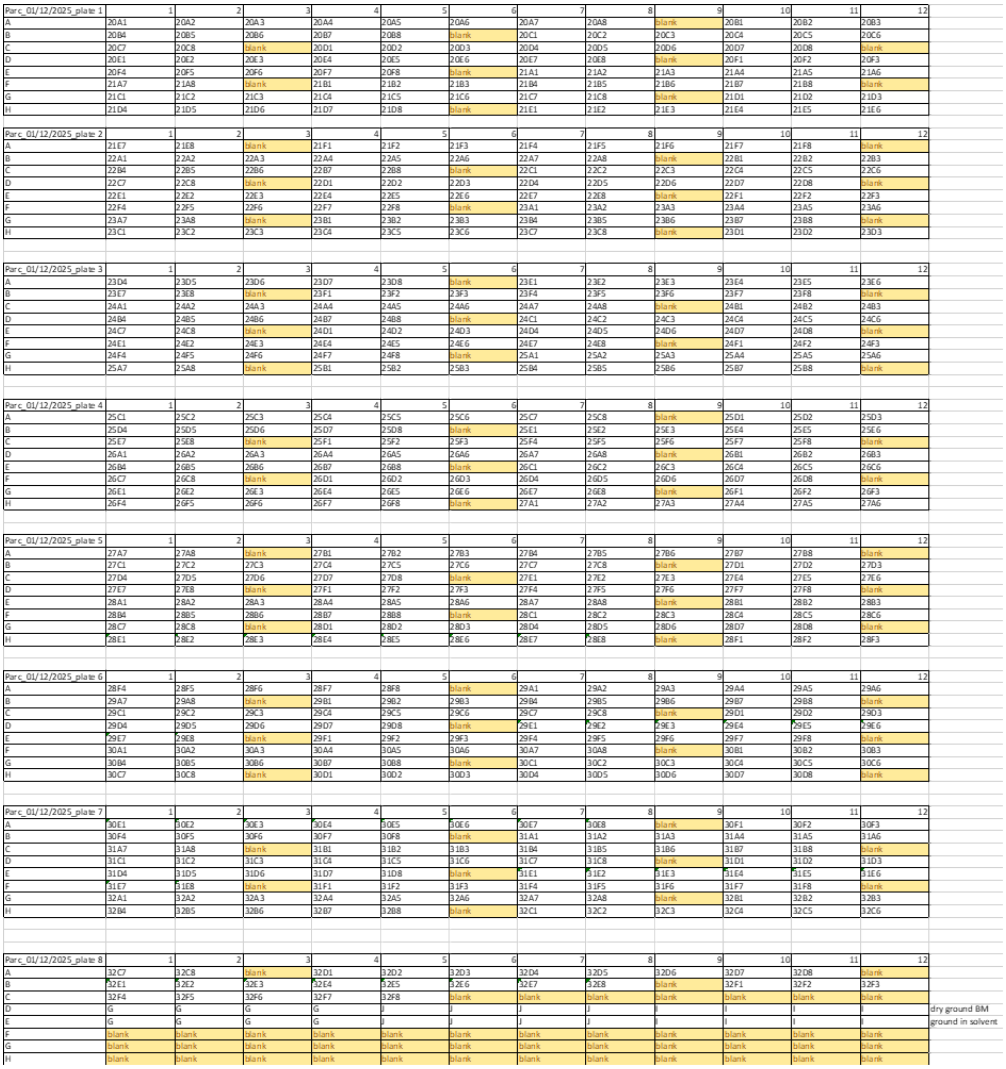
2 Seal the microplate with parafilm and use a tape to stick the microplate to the holder. Then quench the microplate in liquid N_2 for 00:05:00 . 5m

3 After taking out from liquid N_2, seal the microplate with parafilm and store the microplate in freezer -80 °C before further use.

Transfer samples from microplate into micronic tubes

4h 5m

4 Design the micronic plate plan in excel. Each 96 micronic plate contains at least two blank tubes. One blank tube can be replaced by QC during injection. Label the plate on the right side of the rack: “Project_date, number of the plate” e.g. "Parc_12/2025, 1"



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D	G	G	G	G	I	I	I	I	I	I	I	I	dry ground BM
E	G	G	G	G	I	I	I	I	I	I	I	I	ground in solvent
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H	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	

Exemple of micronic 96-well plate plan

5 Weight empty micronic 1.1 mL tubes (NBS MP32033L, without caps) and put the tubes at 96 well micronic rack. Close the lid of the rack before further use.

1.1 mL micronic tube

Micronic 96-well rack

6 Add 200 µL cold EtOH (100%, Grade UPLC) in each well of 48 well plate.

7 Place the plate in a beaker which contained ice water. Then, put the beaker in sonication for 00:05:00 . Don't place the plate directly in the sonication bath, as the power of sonication is not even which can cause well to well contamination. 5m

Ultra-sonication bath: microplate in a beaker contained ice cold water

8 After sonication, using pipette to wash the discs and transfer the EtOH with periphyton into pre-weighted micronic tubes.

9 Dry EtOH by using GENEVAC (Hydro mode), until no more liquid and the sample is completely dry. Probably it will take more than 04:00:00 . If you will using GENEVAC to dry samples in the next day after adding EtOH, put the samples in 4 °C . If you will dry the samples a few days later, put the samples in -20 °C . 4h

10 After GENEVAC, measure the weight of tubes plus dry samples (without caps). Avoid putting the plate on ice during the measurement of dry weight as ice can increase humidity then affect the dry weight measurement.

11 Add one metal bead into each micronic tube for sample grinding before close the cap.

12 Store the micronic plates in -80 °C before metabolites extraction.

Protocol references

Lin Zi, Zahrasadat Alavikakhki, Mélissa Eon, Chloé Bonnineau, Nicolas Creusot 2026. Periphyton growing and sampling in situ or in mesocosm for metabolomic analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l2qjd4l1y/v2

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