Jan 08, 2026 Version 1

Sample Preparation for Automated Extraction in Periphyton Metabolomic Analysis V.1

DOI
https://dx.doi.org/10.17504/protocols.io.81wgbwjoogpk/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.81wgbwjoogpk/v1

Protocol Citation: Lin Zi, Mélissa EON, Nicolas Creusot 2026. Sample Preparation for Automated Extraction in Periphyton Metabolomic Analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbwjoogpk/v1

Protocol status: Working
We use this protocol and it's working

Created: December 05, 2025

Last Modified: January 08, 2026

Protocol Integer ID: 234281

Keywords: chemical risk assessment, periphyton, metabolomics, sample preparation, automated extraction, robotic extraction in periphyton metabolomic, periphyton metabolomic analysis this protocol, periphyton metabolomic analysis, periphyton metabolomic, metabolomic, sample preparation for automated extraction, extraction, sample preparation process, automated robotic extraction, using micronic tube, procedure

Funders Acknowledgements:
PARC: Partnership for the Assessment of Risks from Chemicals
Grant ID: 101057014
Bordeaux Metabolome Platform
Grant ID: https://doi.org/10.15454/1.5572412770331912E12
MetaboHUB
Grant ID: ANR-11-INBS-0010
MetaboHUB
Grant ID: ANR-21-ESRE-0035

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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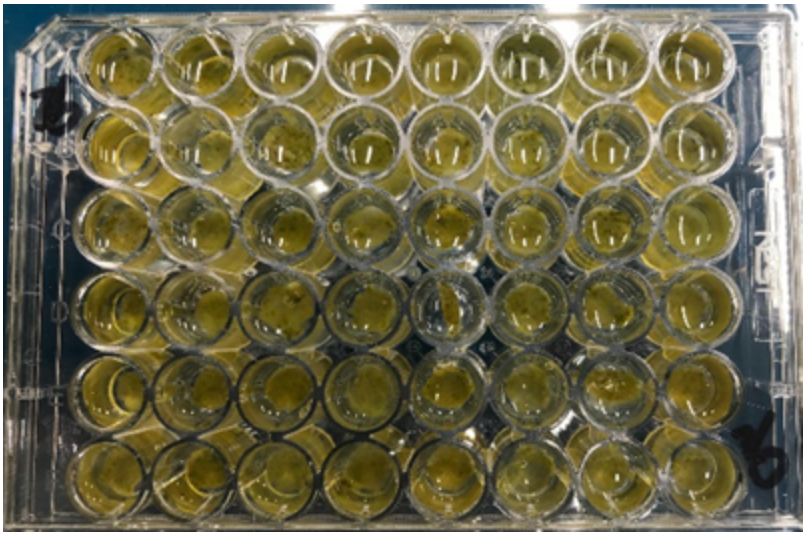

Periphyton grown on 9-mm glass discs in 48-well plate

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"

Parc_01/12/2025_plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	20A1	20A2	20A3	20A4	20A5	20A6	20A7	20A8	blank	20B1	20B2	20B3
B	20B4	20B5	20B6	20B7	20B8	blank	20C1	20C2	20C3	20C4	20C5	20C6
C	20C7	20C8	blank	20D1	20D2	20D3	20D4	20D5	20D6	20D7	20D8	blank
D	20E1	20E2	20E3	20E4	20E5	20E6	20F7	20E8	blank	20F1	20F2	20F3
E	20F4	20F5	20F6	20F7	20F8	blank	21A1	21A2	21A3	21A4	21A5	21A6
F	21A7	21A8	blank	21B1	21B2	21B3	21B4	21B5	21B6	21B7	21B8	blank
G	21C1	21C2	21C3	21C4	21C5	21C6	21C7	21C8	blank	21D1	21D2	21D3
H	21D4	21D5	21D6	21D7	21D8	blank	21E1	21E2	21E3	21E4	21E5	21E6

Parc_01/12/2025_plate 2	1	2	3	4	5	6	7	8	9	10	11	12
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B	22A1	22A2	22A3	22A4	22A5	22A6	22A7	22A8	blank	22B1	22B2	22B3
C	22B4	22B5	22B6	22B7	22B8	blank	22C1	22C2	22C3	22C4	22C5	22C6
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E	22E1	22E2	22E3	22E4	22E5	22E6	22E7	22E8	blank	22F1	22F2	22F3
F	22F4	22F5	22F6	22F7	22F8	blank	23A1	23A2	23A3	23A4	23A5	23A6
G	23A7	23A8	blank	23B1	23B2	23B3	23B4	23B5	23B6	23B7	23B8	blank
H	23C1	23C2	23C3	23C4	23C5	23C6	23C7	23C8	blank	23D1	23D2	23D3

Parc_01/12/2025_plate 3	1	2	3	4	5	6	7	8	9	10	11	12
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B	23E7	23E8	blank	23F1	23F2	23F3	23F4	23F5	23F6	23F7	23F8	blank
C	24A1	24A2	24A3	24A4	24A5	24A6	24A7	24A8	blank	24B1	24B2	24B3
D	24B4	24B5	24B6	24B7	24B8	blank	24C1	24C2	24C3	24C4	24C5	24C6
E	24C7	24C8	blank	24D1	24D2	24D3	24D4	24D5	24D6	24D7	24D8	blank
F	24E1	24E2	24E3	24E4	24E5	24E6	24E7	24E8	blank	24F1	24F2	24F3
G	24F4	24F5	24F6	24F7	24F8	blank	25A1	25A2	25A3	25A4	25A5	25A6
H	25A7	25A8	blank	25B1	25B2	25B3	25B4	25B5	25B6	25B7	25B8	blank

Parc_01/12/2025_plate 4	1	2	3	4	5	6	7	8	9	10	11	12
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B	25D4	25D5	25D6	25D7	25D8	blank	25E1	25E2	25E3	25E4	25E5	25E6
C	25E7	25E8	blank	25F1	25F2	25F3	25F4	25F5	25F6	25F7	25F8	blank
D	26A1	26A2	26A3	26A4	26A5	26A6	26A7	26A8	blank	26B1	26B2	26B3
E	26B4	26B5	26B6	26B7	26B8	blank	26C1	26C2	26C3	26C4	26C5	26C6
F	26C7	26C8	blank	26D1	26D2	26D3	26D4	26D5	26D6	26D7	26D8	blank
G	26E1	26E2	26E3	26E4	26E5	26E6	26E7	26E8	blank	26F1	26F2	26F3
H	26F4	26F5	26F6	26F7	26F8	blank	27A1	27A2	27A3	27A4	27A5	27A6

Parc_01/12/2025_plate 5	1	2	3	4	5	6	7	8	9	10	11	12
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B	27C1	27C2	27C3	27C4	27C5	27C6	27C7	27C8	blank	27D1	27D2	27D3
C	27D4	27D5	27D6	27D7	27D8	blank	27E1	27E2	27E3	27E4	27E5	27E6
D	27E7	27E8	blank	27F1	27F2	27F3	27F4	27F5	27F6	27F7	27F8	blank
E	28A1	28A2	28A3	28A4	28A5	28A6	28A7	28A8	blank	28B1	28B2	28B3
F	28B4	28B5	28B6	28B7	28B8	blank	28C1	28C2	28C3	28C4	28C5	28C6
G	28C7	28C8	blank	28D1	28D2	28D3	28D4	28D5	28D6	28D7	28D8	blank
H	28E1	28E2	28E3	28E4	28E5	28E6	28E7	28E8	blank	28F1	28F2	28F3

Parc_01/12/2025_plate 6	1	2	3	4	5	6	7	8	9	10	11	12
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H	30C7	30C8	blank	30D1	30D2	30D3	30D4	30D5	30D6	30D7	30D8	blank

Parc_01/12/2025_plate 7	1	2	3	4	5	6	7	8	9	10	11	12
A	30E1	30E2	30E3	30E4	30E5	30E6	30E7	30E8	blank	30F1	30F2	30F3
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C	31A7	31A8	blank	31B1	31B2	31B3	31B4	31B5	31B6	31B7	31B8	blank
D	31C1	31C2	31C3	31C4	31C5	31C6	31C7	31C8	blank	31D1	31D2	31D3
E	31D4	31D5	31D6	31D7	31D8	blank	31E1	31E2	31E3	31E4	31E5	31E6
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G	32A1	32A2	32A3	32A4	32A5	32A6	32A7	32A8	blank	32B1	32B2	32B3
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Parc_01/12/2025_plate 8	1	2	3	4	5	6	7	8	9	10	11	12	
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B	32E1	32E2	32E3	32E4	32E5	32E6	32E7	32E8	blank	32F1	32F2	32F3	
C	32F4	32F5	32F6	32F7	32F8	blank	blank	blank	blank	blank	blank	blank	
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
F	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	
G	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	blank	
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

Nicolas Creusot, Mélissa Eon, Lin Zi. 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

Lin Zi, Mélissa ME EON. Contamination procedure for chemical risk assessment in periphyton.**protocols.io** https://protocols.io/view/contamination-procedure-for-chemical-risk-assessme-hgxyb3xpx