Nov 05, 2025

2-in-1 metabolite and protein extraction method for plant multi-omics

DOI
https://dx.doi.org/10.17504/protocols.io.q26g71j29gwz/v1

Qijie Guan[1], Tahmina Akter[1], Sixue Chen[1]

[1]University of Mississippi

Qijie Guan
University of Mississippi

DOI: https://dx.doi.org/10.17504/protocols.io.q26g71j29gwz/v1

Protocol Citation: Qijie Guan, Tahmina Akter, Sixue Chen 2025. 2-in-1 metabolite and protein extraction method for plant multi-omics. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g71j29gwz/v1

Manuscript citation:
10.1002/pmic.70068

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

Created: September 16, 2024

Last Modified: November 05, 2025

Protocol Integer ID: 107655

Keywords: proteins from the same plant sample, extracting metabolite, protein extraction method, protein extraction, same plant sample, lyophilization improved efficiency, protein

Funders Acknowledgements:
National Science Foundation
Grant ID: 2320251
Plant Genome Research Program
Grant ID: 2318746

Disclaimer

Please cite the paper (https://doi.org/10.1002/pmic.70068) if you use this protocol for your publication. Citation: Q. Guan, T. Akter, Y. Singh, B. Tan, and S. Chen, " Lyophilization Improved Efficiency in 2-in-1 Metabolite and Protein Extraction for Plant Multi-Omics." *PROTEOMICS* (2025): e70068. https://doi.org/10.1002/pmic.70068

Abstract

This protocol is a method for extracting metabolites and proteins from the same plant samples, and is the protocol used in manuscript entitled "Lyophilization Improved Efficiency in 2-in-1 Metabolite and Protein Extraction for Plant Multi-Omics", https://doi.org/10.1002/pmic.70068

Materials

Chemicals:

- Metabolite extraction solvent I -20 °C : acetonitrile: isopropanol: water (3:3:2)
- Metabolite extraction solvent II -20 °C : acetonitrile: water (1:1)
- Metabolite extraction solvent III -20 °C : 80% methanol
- Protein lysis buffer (4 °C)
- 100 µM 10-camphorsulfonic acid (10-CA)
- 100 µM lidocaine
- BSA standard

Equipment:

- Liquid nitrogen
- Tubes (1.5 mL centrifuge tubes, 10 mL centrifuge tubes, Geno Grinder tubes)
- Geno/Grinder HG-400
- Beads (washed with 100% methanol and fully dried)
- Research ice
- Balance
- Sonicator
- Speedvac
- Centrifuge (2 mL rotor)

Sample Preparation

2h 10m

1 **Sample collection** 3m

Collect plant material using a humidity chamber, weigh 150 mg for each 2 mL screw cap tube (Cole-Parmer, Vernon Hills, IL, USA), add a 6 mm stainless steel grinding ball (Cole-Parmer, Vernon Hills, IL, USA). Put tubes in liquid nitrogen immediately.

Safety information

Liquid nitrogen is extremely cold and can be hazardous if not handled properly.

2 **Sample vacuum dried** 2h

Vacuum drying samples with Savant SPD1010 SpeedVac Concentrator System (Thermo Scientific,
Asheville, NC, USA).

Equipment

Savant SPD1010 SpeedVac Concentrator System	NAME
Savant	BRAND
SPD1010	SKU

3 **Add internal standards** 2m

For each sample tube, add 3 internal standards:

- 10 µL of 100 µM lidocaine (metabolomics, positive mode)
- 10 µL of 100 µM 10-camphorsulfonic acid (10-CA, metabolomics, negative mode)
- 4 µL of 1 µg/µL Bovine Serum Albumin Fraction V (BSA, proteomics)

4 **Sample homogenizes** 5m

For each tube, add a 6 mm stainless steel grinding ball (Cole-Parmer, Vernon Hil ls, IL, USA). Freeze the tube with liquid nitrogen, homogenize with homogenizer (HG-400, Cole-Parmer, Vernon Hills, IL, USA) for 1000 rpm, 00:02:00. Add liquid nitrogen to the clamp to remain tubes cold.

Equipment

Cole-Parmer SamplePrep HG-400 MiniG® Homogenizer	NAME
homogenizer	TYPE
Cole-Parmer	BRAND
EW-04500-08	SKU
https://www.coleparmer.com/i/cole-parmer-sampleprep-hg-400-minig-homogenizer-2-to-50-ml-115-230-vac-50-60hz/0450008	LINK

Metabolite extraction

45m

5 Add 800 µL pre-cooled (-20 °C) metabolite extraction buffer I (acetonitrile: isopropanol: water, 3:3:2) with 0.1 mM PMSF to the tube. Remove the bead in the tube with magnetic sticker. Vortex 30 seconds, sonicate in ice water for 5 min, centrifuge 15000 x g, 4°C, 00:05:00 .

5m

Safety information

Phenylmethylsulfonyl fluoride (PMSF) is a serine protease inhibitor. It is highly reactive and hazardous, requiring careful handling. **Use Gloves, Eye protection, Lab coat, and Respirator.**

6 Pipetting supernatant to a new 1.5 mL centrifuge tube A. The 1.5 mL supernatant tube A should be kept in a -20 °C freezer.

7 Add 800 µL pre-cooled (-20 °C) metabolite extraction buffer II (acetonitrile: water, 1:1) with 0.1 mM PMSF to the pellet. Vortex 30 seconds, sonicate in ice water for 5 min, centrifuge 15000 x g, 4°C, 00:05:00 .

5m

Safety information

Phenylmethylsulfonyl fluoride (PMSF) is a serine protease inhibitor. It is highly reactive and hazardous, requiring careful handling. **Use Gloves, Eye protection, Lab coat, and Respirator.**

8 Pipetting supernatant to a new 1.5 mL centrifuge tube B. The 1.5 mL supernatant tube B should be kept in a -20 °C freezer.

9 Add 800 µL pre-cooled (-20 °C) metabolite extraction buffer III (80% methanol) with 0.1 mM PMSF to the pellet. Vortex 30 seconds, sonicate in ice water for 5 min, centrifuge 15000 x g, 4°C, 00:05:00 . 5m

Safety information

Phenylmethylsulfonyl fluoride (PMSF) is a serine protease inhibitor. It is highly reactive and hazardous, requiring careful handling. **Use Gloves, Eye protection, Lab coat, and Respirator.**

10 Pipetting supernatant to a new 1.5 mL centrifuge tube C. The remaining residue will be used in protein extraction (step 13)

11 Vacuum dry the three collected tubes, combine extracts from the same sample together after 1 h of vacuum drying. Pipet the sample in B, C tubes to A tube, so that all metabolites will be transferred and combined in one tube for drying down.

12 Once dried down, the residue is the metabolite section.

Protein extraction 1h

13 Vacuum dry metabolite extraction residue (from step 10) for 5 min to evaporate methanol remaining.

14 Add 200 µL protei n Lysis solubilization solution (7 M urea, 2 M thiourea, 4% CHAPS, 10 mM DTT and 0.1 mM PMSF).

Safety information

Phenylmethylsulfonyl fluoride (PMSF) is a serine protease inhibitor. It is highly reactive and hazardous, requiring careful handling. **Use Gloves, Eye protection, Lab coat, and Respirator.**

15 Vortex in cold room (4 °C) for 30 min, sonicate in ice water for 5 min, centrifuge 15000 x g, 4°C, 00:15:00 . 15m

16 Collect the supernatant, this is the protein solvent.