PREOMICS

iST 8x

Plant Tissue

Introduction

Sample preparation is one of the essential steps of bottom-up proteomics. The PreOmics® iST sample preparation kit is designed to assist researchers achieving best results with few sample preparation steps and little hands-on time. For specific recommendations and optimization, visit www.preomics.com/faq or contact info@preomics.com.

Kit Contents

The kit includes all essential components for proteomic sample preparation: denaturing, reducing, and alkylating agents, enzymes, cartridges, and wash buffers for peptide cleanup.

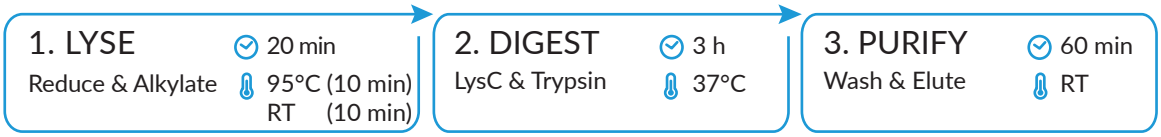
Component	Cap	Quantity	Buffer Properties				Description	Storage
iST 8x (P.O.00001)			Organic	Acidic	Basic	Neutral		
DIGEST		2x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND		1x 2 mL				●	For reconstituting lyophilized proteolytic enzymes.	RT
LYSE		1x 1 mL			●		For denaturing, reducing, and alkylating proteins.	RT
STOP		1x 1 mL	●	●			For stopping enzymatic activity.	RT
WASH 1		1x 2 mL	●	●			For removing hydrophobic contaminants.	RT
WASH 2		1x 2 mL		●			For removing hydrophilic contaminants.	RT
ELUTE		1x 2 mL	●		●		For eluting peptides from the cartridge.	RT
LC-LOAD		1x 1 mL		●			For loading peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		8x					Cartridges with SPE sorbent for cleaning up peptides from 1–100 µg protein starting material.	RT
WASTE TUBE		8x					2 mL tube for collecting waste after washing steps.	RT
COLLECTION TUBE		8x					1.5 mL tube for collecting peptides after elution.	RT
ADAPTER		8x					Enables a cartridge to be placed into a tube.	RT
Additional reagents, kits and instruments from PreOmics:								
WASH 0 (P.O.00095)		10 mL	●	●			Cleans peptides from phytochemicals. Please order 1x WASH 0 buffer 10 mL (P.O.00095) from PreOmics, or ask for the buffer recipe at info@preomics.com.	RT

Pre-Requisites

Common lab equipment is required for the sample preparation.

Equipment	Quantity and Description
PIPETTE	Careful sample handling and pipetting reduces sample contaminations and improves quantification.
SAMPLE	Plant tissue containing 1–100 µg protein starting material and cryomilled. Protein content strongly depends on tissue type. Visit our FAQ for more information on tissue starting amounts. Alternatively, use BeatBox® to homogenize intact plant material. Contact PreOmics for an adapted protocol.
REACTION TUBE	1.5 mL microcentrifuge low protein binding tubes are recommended (e.g., Eppendorf Protein LoBind® Tubes, catalogue number EU: 0030108116, US: 022431081).
HEATING SHAKER	Two heating shakers for tubes are recommended for protein denaturation and digestion.
CENTRIFUGE	Benchtop centrifuge for 1.5 or 2 mL tubes is required for peptide loading, washing, and elution.
SONICATOR	For tissue homogenization and lysis (e.g., Diagenode Bioruptor®).
VACUUM EVAPORATOR	To evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

Procedure



Method

1. LYSE

1.1. Add 100 µL **LYSE** to 1–100 µg of protein sample in a REACTION TUBE, place it in a pre-heated HEATING SHAKER (95°C; 1,000 rpm; 10 min). *NOTE1*

1.2. Optional: Spin down droplets (RT; max. 300 rcf; 10 sec).

1.3. Shear the sample in a SONICATOR (10 cycles; 30 sec ON/OFF).

1.4. Optional: Spin down droplets (RT; max. 300 rcf; 10 sec).

2. DIGEST

2.1. Optional: Spin down lyophilized enzyme mix in the **DIGEST** tube (RT; max. 300 rcf; 10 sec).

2.2. Add 210 µL **RESUSPEND** to **DIGEST** (1 tube for 4 reactions), shake (RT; 500 rpm; 10 min) and pipette up/down. *NOTE2*

2.3. Add 50 µL **DIGEST** to REACTION TUBE and place it in a pre-heated HEATING SHAKER (37°C; 500 rpm; 3 h).

2.4. Add 100 µL **STOP** to REACTION TUBE (precipitation may occur), shake (RT; 500 rpm; 1 min) and pipette up/down.*SP*

2.5. Spin sample in CENTRIFUGE (16,000 rcf; 1 min).

2.6. Assemble **ADAPTER** and **CARTRIDGE** to the top of **WASTE TUBE**. Label all tubes and transfer supernatant to the **CARTRIDGE**.

3. PURIFY

3.1. Centrifuge the **CARTRIDGE** (RT; 3,800 rcf; 1–3 min). If needed, adjust time to ensure complete flow-through.

3.2. Add 200 µL **WASH 0** to **CARTRIDGES**, repeat step 3.1.

3.3. Add 200 µL **WASH 1** to **CARTRIDGE**, repeat step 3.1.

3.4. Add 200 µL **WASH 2** 🟢 to **CARTRIDGE**, repeat step 3.1. *SP*

3.5. Transfer assembled **ADAPTER** and **CARTRIDGE** onto clean **COLLECTION TUBE**. Discard **WASTE TUBE**. Label all tubes.

3.6. Add 100 µL **ELUTE** 🔴 to **CARTRIDGE**, repeat step 3.1, keep flow-through in **COLLECTION TUBE**.

3.7. Repeat step 3.6, keep flow-through in the same **COLLECTION TUBE**.

3.8. Discard **CARTRIDGE** and place **COLLECTION TUBE** in a VACUUM EVAPORATOR (45°C; until completely dry). *SP*

3.9. Reconstitute peptides by adding **LC-LOAD** ◯ to **COLLECTION TUBE.** Adjust the volume according to specific requirements. For example, add 50 µL **LC-LOAD** to 100 µg protein starting material.

3.10. Sonicate **COLLECTION TUBE** in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min).

3.11. Spin **COLLECTION TUBE** in a CENTRIFUGE (RT; 16,000 rcf; 5 min). Transfer the supernatant to a clean vial and avoid touching the bottom of the **COLLECTION TUBE** during transfer. *NOTE3*

NOTE1 Buffer volumes can be adjusted according to protein starting amounts. Lysis temperature should be between 60–95°C. Visit our FAQ website for more information.

NOTE 2 Resuspended DIGEST can be stored for up to two weeks at 4°C. For longer storage periods, visit our FAQ.

NOTE 3 At this point, peptide concentration can be measured, or the sample can be directly injected for LC-MS analysis. Visit our FAQ for recommendations on peptide quantitation assays.

SP - Storage Point: At this point, close the peptide containing **COLLECTION TUBE** or **CARTRIDGE** using the silicone lid. Peptides can be frozen at -20°C for two weeks. Dried peptides, prior to reconstitution in **LC-LOAD**, can also be stored long-term at -80°C.

Data analysis

Consider the following as fixed modifications in your database search:

MODIFICATION	DESCRIPTION	COMPOSITION	SPECIFICITY	MASS	UNIMOD #
ALKYLATION	Carbamidomethyl on cysteine	C_2H_3NO	[C]	+57Da	4

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