iST Sample Preparation Kit (8 reactions)
PROTOCOL - Plant Tissue

INTRODUCTION

Sample preparation is one of the essential steps of bottom-up proteomics. The PreOmics sample preparation kit is designed to assist you achieving best results with few sample preparation steps and little hands-on time. For sample-specific protocols and optimization contact us or visit our web-site at www.preomics.com.

KIT CONTENTS

The kit contains all you need to perform a complete sample preparation. It includes all chemicals to denature, reduce and alkylate proteins, as well as the enzymes to perform a tryptic digestion and a final cleanup.

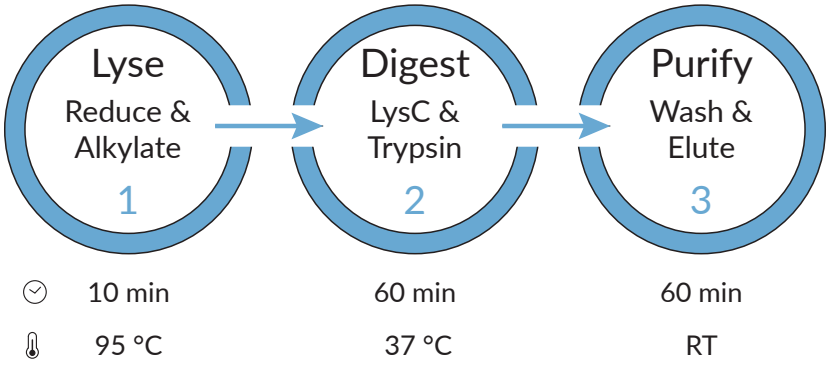
Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Volatile		
DIGEST	red	2x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	yellow	1x 2 mL				✓	Protease reconstitution buffer for enzymes.	RT
LYSE	brown	1x 1 mL			✓		Denatures, reduces and alkylates proteins.	RT
STOP	black	1x 1 mL	✓	✓		✓	Stops the enzymatic activity.	RT
WASH 0	no color	1x 2 mL	✓	✓		✓	Clean up peptides from phytochemicals.	RT
WASH 1	blue	1x 2 mL	✓	✓		✓	Clean up peptides from hydrophobic contaminants.	RT
WASH 2	green	1x 2 mL		✓		✓	Clean up peptides from hydrophilic contaminants.	RT
ELUTE	violet	1x 2 mL	✓		✓	✓	Elutes the peptides from the cartridge.	RT
LC-LOAD	white	1x 1 mL		✓		✓	Load peptides on reversed-phase LC-MS column.	RT
CARTRIDGES		8x					Cartridge for 1 to 100 µg protein starting material.	RT
WASTE		8x					Tube for collecting waste after washing steps.	RT
COLLECTION		8x					Tube for collecting peptides after elution.	RT
ADAPTER		8x					Enables a cartridge to be placed into a tube.	RT
CAP		8x					Cap to (optionally) close the cartridge`s bottom.	RT

PRE-REQUISITES

Common lab equipment is required for the sample preparation.

EQUIPMENT	DESCRIPTION
PIPETTE	Careful sample handling and pipetting reduces contaminations and improves quantification.
TUBES	Only use 1.5 mL Eppendorf LoBind tubes to avoid polymer leaching and reduce protein binding to tube walls.
SAMPLE	Cryomilled plant material (or other means of cryogenic grinding with liquid nitrogen).
HEATING BLOCK	Two heating blocks are recommended to support protein denaturation and digestion.
CENTRIFUGE	Eppendorf tube centrifuges are necessary for loading, washing and elution.
SONICATOR	Ultrasonication guarantees efficient tissue disruption and homogenization (e.g. Diagenode Bioruptor®).
SPEED-VAC	Vacuum manifolds evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

PROCEDURE



Material: Cryomilled plant material
Quantity: 1-100 µg protein starting material

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PROTOCOL

1. LYSE

1.1. Transfer 1-100 µg of cryomilled plant material into a clean 1.5 mL Eppendorf LoBind tube, add 100 µL **LYSE** ●.

1.2. Place sample in a HEATING BLOCK (95°C; 1,000 rpm; 10 min). *NOTE 1*

1.3. Shear 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. Add 210 µL **RESUSPEND** ○ to **DIGEST** ● (1 tube for 4 reactions), shake (RT; 500 rpm; 10 min), pipette up/down.

2.2. Add 50 µL **DIGEST** ● to sample and place it in a pre-heated HEATING BLOCK (37 °C; 500 rpm; 1-3 hours).

2.3. Add 100 µL **STOP** ● to sample (precipitation may occur), shake (RT; 500 rpm; 1 min), pipette up/down. *SP*

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

3. PURIFY

3.1. Use **ADAPTER** to place **CARTRIDGE** in **WASTE** tube. Label all tubes.

3.2. Transfer supernatant from 2.4. to **CARTRIDGE**. Be careful not to damage the bottom layer of **CARTRIDGE**.

3.3. Spin **CARTRIDGE** in a CENTRIFUGE (3,800 rcf; 1-3 min). If needed, adjust values to ensure complete flow-through.

3.4. Add 200 µL **WASH 0** (no color) to **CARTRIDGE**, repeat step 3.3., discard flow-through.

3.5. Add 200 µL **WASH 1** ● to **CARTRIDGE**, repeat step 3.3., discard flow-through.

3.6. Add 200 µL **WASH 2** ● to **CARTRIDGE**, repeat step 3.3., discard flow-through. *SP*

3.7. Use **ADAPTER** to place **CARTRIDGE** in a fresh **COLLECTION** tube. Label all tubes.

3.8. Add 100 µL **ELUTE** ● to **CARTRIDGE**, repeat step 3.3., keep flow-through in **COLLECTION** tube.

3.9. Repeat step 3.8., keep flow-through in the same **COLLECTION** tube.

3.10. Discard **CARTRIDGE** and place **COLLECTION** tube in a SPEED-VAC (45 °C; until completely dry).

3.11. Add **LC-LOAD** ○ to **COLLECTION** tube. Aim for 1 g/L concentration (e.g. 100 µL to 100 µg protein starting material).

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

NOTE 1 Volumes of buffers can be adjusted according to protein starting amounts.

Lysis temperature should be between 60-95 °C. Visit our FAQ website for more information: www.preomics.com/faq.

SP - Storage Point: At this point, close the peptide containing tube or **CARTRIDGE** (use a **CAP** for bottom).

Peptides can be frozen at -20 °C. Storage of peptides should not exceed two weeks at -20 °C.

For extended storage, finish the protocol and store 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]	+57 Da	4

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