PREOMICS

iST 96x HT

P.O.00150; P.O.00245

Pelleted cells & precipitated protein

Introduction

Sample preparation is one of the essential steps of bottom-up proteomics. The PreOmics® iST sample preparation kit is designed to streamline this process, enabling researchers to achieve optimal results with reduced steps and hands-on time. For sample-specific protocols and optimization, visit www.preomics.com/resources 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.

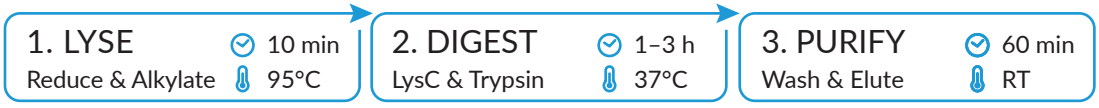
Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Neutral		
DIGEST		24x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND		1x 20 mL				●	For reconstituting lyophilized proteolytic enzymes.	RT
LYSE		1x 20 mL			●		For denaturing, reducing, and alkylating proteins.	RT
STOP		1x 15 mL	●	●			For stopping enzymatic activity.	RT
WASH 1		1x 25 mL	●	●			For removing hydrophobic contaminants.	RT
WASH 2		1x 25 mL		●			For removing hydrophilic contaminants.	RT
ELUTE		1x 25 mL	●		●		For eluting peptides from the cartridge.	RT
LC-LOAD		1x 25 mL		●			For loading peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		96x					Cartridges with SPE sorbent for peptide purification from 1–100 µg protein starting material. Racked in adapter plate and sealed with silicone mat.	RT
WASTE PLATE		1x					Deep well plate for collecting waste after washes.	RT
COLLECTION PLATE		1x					LoBind® plate for collecting peptides after elution. The plate has a max. working volume of 150 µL, and can be used after validating the workflow for elution with lower buffer volumes. Alternatively, use ELUTE PLATE (see Pre-Requisites section below).	RT
ADAPTER PLATE		1x					Enables cartridges to be placed on top of 96-well plates.	RT
ADAPTER		8x					Enables a cartridge to be placed into a tube.	RT

Pre-Requisites

Common lab equipment is required for the sample preparation.

Equipment	Quantity and Description
PIPETTE	Careful sample handling and pipetting reduce contamination and improve quantification.
REACTION PLATE	Samples may be handled in any reaction vessel >450 µL, but a 96 deep well plate is recommended (e.g., Eppendorf Deepwell plate 96/500 µL Protein LoBind®, catalogue number EU: 0030504100, US: 951032107).
SEALING MAT	Prevents sample contamination and evaporation (e.g., Eppendorf Sealing Mat, catalogue number EU&US: 0030127978).
SAMPLE	Pelleted cells or precipitated protein (1–100 µg protein starting material; max. 10 µL sample volume). For higher sample volumes, visit our FAQs. For other sample types, contact PreOmics for adapted protocols.
96-WELL PLATES	96 deep well & 96 well skirted plates to balance WASTE & COLLECTION PLATES in centrifuge.
HEATING SHAKER	Two heating shakers for multi-well plates are recommended to support protein denaturation and digestion.
CENTRIFUGE	Swing-bucket centrifuges are required for peptide loading, washing, and elution.
SONICATOR or NUCLEASE	If the sample is viscous, use a sonicator to shear DNA or add nuclease (e.g., Benzonase®) to degrade both DNA and RNA. Visit our FAQ for more information.
ELUTE PLATE	96 deep well plate with >250 µL for elution of peptides from cartridge (e.g., Eppendorf Deepwell plate 96/500 µL Protein LoBind®, catalogue number EU: 0030504100, US: 951032107).
VACUUM EVAPORATOR	To evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

Procedure



Method

Critical Note: For automation processes, only plates with low protein binding properties should be used as buffer reservoirs to avoid polymer contamination. Contact us at info@preomics.com for advice on buffer and plasticware usage with liquid handling platforms.

1. LYSE

1.1. Add 50 µL **LYSE** to 1–100 µg of protein sample in a REACTION PLATE, close plate with a SEALING MAT and place the plate 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. If sample is viscous, use a SONICATOR or add NUCLEASE.

1.4. Allow samples to cool to room temperature.

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 each well, close REACTION PLATE with a SEALING MAT and place the plate in a pre-heated HEATING SHAKER (37°C; 500 rpm; 1–3 h).

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

2.5. Place **ADAPTER PLATE** with CARTRIDGE on **WASTE PLATE**. Label plate and wells, and transfer the sample to the **CARTRIDGE**. *NOTE3*

3. PURIFY

3.1. Spin **CARTRIDGE** in a CENTRIFUGE (2,250 rcf; 1–3 min). If needed, adjust time to ensure complete flow-through.

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

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

3.4. Discard **WASTE PLATE**. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of the ELUTE PLATE. Label plate and wells.
Alternatively, use the supplied **COLLECTION PLATE** after validating the workflow for elution with lower buffer volumes. See "Kit Contents" for details.

3.5. Add 100 µL **ELUTE** to **CARTRIDGE**, repeat step 3.1, keep flow-through in ELUTE PLATE.

3.6. Repeat step 3.5, keep flow-through in the same ELUTE PLATE.

3.7. Discard **CARTRIDGE** and place ELUTE PLATE in a VACUUM EVAPORATOR (45°C; until completely dry). *SP*

3.8. Reconstitute peptides by adding **LC-LOAD** to ELUTE PLATE. Adjust the volume according to specific requirements.
For example, add 50 µL **LC-LOAD** to 100 µg protein starting material.

3.9. Sonicate ELUTE PLATE in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min).

3.10. Spin plate in a CENTRIFUGE as follows:

- User-provided ELUTE PLATE: RT; maximum rcf recommended by manufacturer; 5–15 min.
- **COLLECTION PLATE**: RT; 2,250 rcf; 15 min.

Transfer the supernatant to a clean plate and avoid touching the bottom of the well during transfer. *NOTE4*

NOTE1 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.

NOTE2 Resuspended DIGEST can be stored for up to two weeks at 4°C. For longer storage periods, visit our FAQ.

NOTE3 Alternatively, use **ADAPTERS** to place single **CARTRIDGES** on top of 2 mL tubes.
Refer to the corresponding iST 8x kit protocol for subsequent steps.

NOTE4 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. The silicone mat provided with the kit is incompatible with autosamplers.

SP - Storage Point: At this point, close the peptide containing plate or **CARTRIDGE** using the silicone mat.
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

For answers to frequently asked questions, please visit our FAQ page at www.preomics.com/faq.

For our General Terms and Conditions, refer to www.preomics.com.

For trademark information, visit www.preomics.com/legal/trademarks.