PREOMICS

iST-NHS 4x

P.O.00062; P.O.00188

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 iST-NHS kit includes all essential components for proteomic sample preparation, compatible with chemical labeling: denaturing, reducing, and alkylating agents, enzymes, cartridges, and wash buffers for peptide cleanup.

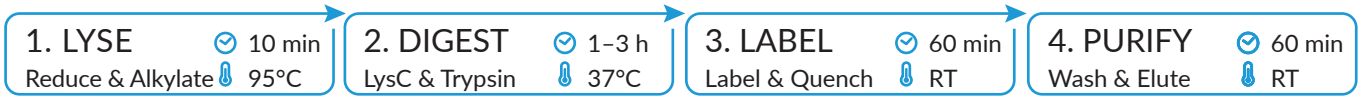
Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Neutral		
DIGEST		1x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND		1x 2 mL				●	For reconstituting lyophilized proteolytic enzymes.	RT
LYSE-NHS		1x 2 mL			●		For denaturing, reducing, and alkylating proteins.	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		4x					Cartridges with SPE sorbent for peptide purification from 1–100 µg protein starting material.	RT
WASTE TUBE		4x					2 mL tube for collecting waste after washing steps.	RT
COLLECTION TUBE		4x					1.5 mL tube for collecting peptides after elution.	RT
ADAPTER		4x					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 reduces contaminations and improves quantification.
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.
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 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 or NUCLEASE	If the sample is viscous, use a sonicator to shear DNA (e.g., Diagenode Bioruptor®) or add nuclease (e.g., Benzonase®) to degrade both DNA and RNA. Visit our FAQ for more information.
VACUUM EVAPORATOR	To evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.
LABELING REAGENT	Labeling reagent (e.g., 400 µg labeling reagent in 41 µL dry acetonitrile for 100 µg peptides).
LABELING BUFFER	Anhydrous acetonitrile, as recommended by the manufacturer.
QUENCHING BUFFER	5% hydroxylamine, as recommended by the manufacturer.

Procedure



Method

1. LYSE

1.1. Add 50 µL **LYSE-NHS** 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. If the sample is viscous, use a SONICATOR or add NUCLEASE.

1.4. Allow samples to cool down 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 REACTION TUBE and place it in a pre-heated HEATING SHAKER (37°C; 500 rpm; 1–3 h).

3. LABEL

3.1. Resuspend LABELING REAGENT in LABELING BUFFER (e.g., 4:1 ratio of label:peptides).

3.2. Add resuspended LABELING REAGENT to REACTION TUBE, pipette up/down, incubate shaking (RT; 500 rpm; 1 h).

3.3. Add 10 µL QUENCHING BUFFER to REACTION TUBE, and pipette up/down.

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

4. PURIFY

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

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

4.3. Add 200 µL **WASH 1** 🔵 to **CARTRIDGE**, repeat step 4.2.

4.4. Add 200 µL **WASH 2** 🟢 to **CARTRIDGE**, repeat step 4.2. *SP*

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

4.6. Add 100 µL **ELUTE** 🔴 to **CARTRIDGE**, repeat step 4.2, keep flow-through in **COLLECTION TUBE.**

4.7. Repeat step 4.6, keep flow-through in the same **COLLECTION TUBE**.

4.8. Discard **CARTRIDGE** and place **COLLECTION TUBE** in a vacuum evaporator (45°C; until completely dry). *SP*

4.9. 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.

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

4.11. 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 and optimized procedures for chemical labeling.
NOTE2	Resuspended DIGEST can be stored for up to two weeks at 4°C. For longer storage periods, visit our FAQ.
NOTE3	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
ALKYLATION	Specific cysteine modification	$C_6H_{11}NO$	[C]	+113.084Da

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