PREOMICS

iST-S 96x HT

P.O.00259; P.O.00286

Cells & pelleted protein

Introduction

Sample preparation is one of the essential steps of bottom-up proteomics. The PreOmics® iST-S sample preparation kit is designed to streamline this process for low amounts of starting material, enabling researchers to achieve optimal results with reduced steps and hands-on time. For specific recommendations 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-L		4x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND		2x 1 mL				●	For reconstituting lyophilized proteolytic enzymes.	RT
LYSE A		2x 1.4 mL				●	For denaturing, reducing, and alkylating proteins.	RT
LYSE B		2x 1.4 mL				●	For denaturing, reducing, and alkylating proteins.	RT
STOP		2x 25 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		2x 1.4 mL		●			For loading peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		96x					Cartridges with SPE sorbent for peptide purification. 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.	RT
ADAPTER PLATE		1x					Enables cartridges to be placed on top of 96-well plates	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	96-well micro plates are recommended (e.g., Eppendorf LoBind® twin.tec® PCR Plates, catalogue number 0030129.512).
SEALING MAT	Prevents sample contamination and evaporation (e.g., AXYGEN Sealing Mat, product number: AM-96-PCR-RD).
SAMPLE	Pelleted & adherent cells (equivilant to 1–10 µg total protein).
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.
VACUUM EVAPORATOR	To evaporate volatile buffers from the eluate before LC-MS.

Procedure

1. LYSE	10 min	2. DIGEST	60 min	3. PURIFY	60 min
Reduce & Alkylate	80°C	Trypsin & LysC	37°C	Wash & Elute	RT

Method

1. LYSE

1.1. The **LYSE** volume needed per sample is 40 µL. Calculate the total volume of **LYSE** required for your experiment. Prepare the calculated **LYSE** volume by mixing equal volumes of **LYSE-A** and **LYSE-B** in a suitable vessel. Briefly vortex the LYSE mixture.

1.2. Add 40 µL **LYSE** to 1–10 µg of protein sample.

1.3. Place SEALING MAT on the REACTION PLATE and place it on a pre-heated HEATING SHAKER (80°C; 1,000 rpm; 10min).

1.4. Optional: Quick spin to remove condensation from the lid (RT; max. 300 rcf; 10 sec).

1.5. Allow samples to cool down to room temperature.

2. DIGEST

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

2.2. Add 300 µL **RESUSPEND** to **DIGEST-L** (1 tube for 24 reactions) and shake (RT; 500 rpm; 10 min). *NOTE1*

2.3. Add 10 µL **DIGEST-L** to each well.

2.4. Place SEALING MAT on the REACTION PLATE. Place REACTION PLATE on a pre-heated HEATING SHAKER (37°C; 1000 rpm; 1 h)

2.5. Optional: Spin to remove condensation from the lid (RT; max. 300 rcf; 10 sec).

2.6. Add 60 µL **STOP** to each well, place SEALING MAT on the REACTION PLATE and shake (RT; 300 rpm; 1 min).

2.7. Place **CARTRIDGES** in **ADAPTER PLATE** on **WASTE PLATE**.

3. PURIFY

3.1. Optional: Priming before transferring the samples to the **CARTRIDGES** by adding 200 µL **STOP**. Place **CARTRIDGES** in a centrifuge and spin (RT, 1000 rcf for 1 min). Make sure the **CARTRIDGES** do not dry out afterwards and directly continue with step 3.2. *NOTE2*

3.2. Transfer the samples to the **CARTRIDGES.**

3.3. Place CARTRIDGES in a CENTRIFUGE and spin (1,000 rcf; 1 min). If needed, adjust time to ensure complete flow-through.

3.4. Add 100 µL **WASH 1** to the CARTRIDGES and centrifuge (2,250 rcf; 1 min).

3.5. Add 100 µL **WASH 2** to the CARTRIDGES and centrifuge (2,250 rcf; 1 min).

3.6. Discard **WASTE PLATE.** Use **ADAPTER PLATE** to place **CARTRIDGES** on top of the **COLLECTION PLATE**.

3.7. Add 75 µL **ELUTE** to the **CARTRIDGES.** Place **CARTRIDGES** in a CENTRIFUGE and spin (1,000 rcf; 1 min). Keep flow-through in the **COLLECTION PLATE**.

3.8. Add further 75 µL **ELUTE** to the **CARTRIDGES** and centrifuge (1,000 rcf; 1 min). Keep flow-through in the same **COLLECTION PLATE**.

3.9. Discard CARTRIDGES and ADAPTER PLATE and place COLLECTION PLATE in a VACUUM EVAPORATOR (RT; until completely dry). *SP*

3.10. Reconstitute peptides by adding LC-LOAD . *NOTE3*

3.11. Adjust the peptide concentration according to the LC–MS column loading requirements. *NOTE4*

It is recommended to centrifuge the samples prior to LC–MS analysis (RT; 2,250 rcf; 10 min).

NOTE1 For 96 reactions, 960 µL of digest mixture are required, which is close to the available volume (1,200 µL). Liquid reservoirs typically have a high dead volume and may not be suitable for the DIGEST-L pipetting steps.

NOTE2 Priming may increase protein identifications, especially for protein input amounts below 5 µg.

NOTE3 Using 20 µL of LC-LOAD will typically result in a peptide concentration of 0.2 µg/µL for 5 µg and 0.3 µg/µL for 10 µg sample input.

NOTE4 For accurate adjustment of peptide injection amount, a peptide quantification should be performed. It is recommended using a quantitative fluorometric assay (e.g., Pierce Quantitative Fluorometric Peptide Assay Kit, Thermo Scientific™, catalogue number 23290), with an input of 2 µL peptide sample + 8 µL LC-LOAD.

SP - Storage Point: Dried peptides, prior to reconstitution in LC-LOAD, can 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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