PREOMICS

iST-S 12x

P.O.00258; P.O.00285

Pelleted & adherent cells

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		1x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND		1x 1 mL				●	For reconstituting lyophilized proteolytic enzymes.	RT
LYSE A		1x 1 mL				●	For denaturing, reducing, and alkylating proteins.	RT
LYSE B		1x 1 mL				●	For denaturing, reducing, and alkylating proteins.	RT
STOP		4x 1 mL	●	●			For stopping enzymatic activity.	RT
WASH 1		2x 1 mL	●	●			For removing hydrophobic contaminants.	RT
WASH 2		2x 1 mL		●			For removing hydrophilic contaminants.	RT
ELUTE		2x 1.4 mL	●		●		For eluting peptides from the cartridge.	RT
LC-LOAD		1x 1.4 mL		●			For loading peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		12x					Cartridges with SPE sorbent for peptide purification sealed with silicone mat.	RT
WASTE TUBE		12x					2 mL tube for collecting waste after washing steps.	RT
COLLECTION TUBE		12x					1.5 mL tube for collecting peptides after elution.	RT
ADAPTER		12x					Enables a cartridge to be placed into a tube. It can be used for further experiments.	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	Benchtop centrifuges for plates and 1.5/2 mL standard tubes are required.
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).

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 REACTION VESSEL, shake (RT; 300 rpm; 1 min).

2.7. Place **CARTRIDGES** in **WASTE TUBES** by using **ADAPTERS**.

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;1 min). Make sure the **CARTRIDGES** do not dry out afterwards and directly continue with step 3.2. *NOTE1*

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 TUBES.** Place **CARTRIDGES** in **COLLECTION TUBES** using **ADAPTERS**.

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 TUBES**.

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

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

3.10. Reconstitute peptides by adding LC-LOAD . *NOTE2*

3.11. Adjust the peptide concentration according to the LC–MS column loading requirements. *NOTE3*

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

NOTE1 Priming may increase protein identifications, especially for protein input amounts below 5 µg.

NOTE2 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.

NOTE3 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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