PREOMICS

iST-NHS Sample Preparation Kit **96x**

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 assist researchers achieving best results with few sample preparation steps and little hands-on time. For sample-specific protocols and optimization visit www.preomics.com/downloads or contact info@preomics.com.

Kit Contents

The iST-NHS kit provides a streamlined solution for reliable sample preparation compatible with chemical labeling. It includes all chemicals to denature, reduce and alkylate proteins, as well as the enzymes to perform a tryptic digestion and a final peptide cleanup.

Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Volatile		
DIGEST	red	24x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	yellow	4x 2 mL				●	Reconstitutes lyophilized proteolytic enzymes.	RT
LYSE-NHS	orange	12x 2 mL			●		Denatures, reduces and alkylates proteins.	RT
STOP	black	12x 1 mL	●	●		●	Stops the enzymatic activity.	RT
WASH 1	blue	12x 2 mL	●	●		●	Cleans peptides from hydrophobic contaminants.	RT
WASH 2	green	12x 2 mL		●		●	Cleans peptides from hydrophilic contaminants.	RT
ELUTE	pink	12x 2 mL	●		●	●	Elutes the peptides from the cartridge.	RT
LC-LOAD	white	12x 1 mL		●		●	Loads peptides on reversed-phase LC-MS column.	RT
CARTRIDGES		96x					Cartridge for 1 to 100 µg protein starting material.	RT
WASTE PLATE		1x					Deep well plate for collecting waste after washes.	RT
MTP PLATE		1x					LoBind plate for collecting peptides after elution.	RT
ADAPTER PLATE		1x					Enables cartridges to be placed on top of 96w 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 reduces contaminations and improves quantification.
SAMPLE	Pelleted cells or precipitated protein. For other sample types contact PreOmics for adapted protocols.
96 WELL PLATES	96 deep well & 96 well skirted plates to balance WASTE & MTP PLATES in centrifuge.
HEATING BLOCK	Two MTP plate heaters are recommended to support protein denaturation and digestion.
CENTRIFUGE	Swing-bucket centrifuges are required for loading, washing and elution.
SONICATOR	If the sample contains DNA, shear it by sonication (e.g. Diagenode Bioruptor®).
VACUUM EVAPORATOR	Vacuum manifolds 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 & quenching buffer (5% hydroxylamine), as recommended by the manufacturer.

1. LYSE	10 min	2. DIGEST	60 min	3. LABEL	60 min	4. PURIFY	60 min
Reduce & Alkylate	95°C	LysC & Trypsin	37°C	Label & Quench	RT	Wash & Elute	RT

Method

1. LYSE

1.1. Add 50 µL **LYSE-NHS** to 1-100 µg of protein sample, place it in a HEATING BLOCK (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 contains DNA, shear it in a SONICATOR (10 cycles; 30 sec ON/OFF). Let sample cool down to RT.

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). *NOTE2*

3. LABEL

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

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

3.3. Add 10 µL QUENCHING BUFFER (5% hydroxylamine) to sample, pipette up/down.

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

4. PURIFY

4.1. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of **WASTE PLATE**. Label plate and wells.

4.2. Transfer sample to **CARTRIDGE**. Be careful not to damage the bottom layer of the **CARTRIDGE**.

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

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

4.5. Add 200 µL **WASH 2** to **CARTRIDGE**, repeat step 4.3. *SP*

4.6. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of the **MTP PLATE**. Label plate and wells.

4.7. Add 100 µL **ELUTE** to **CARTRIDGE**, repeat step 4.3., keep flow-through in **MTP PLATE**.

4.8. Repeat step 4.7., keep flow-through in the same **MTP PLATE**.

4.9. Discard **CARTRIDGE** and place **MTP PLATE** in a vacuum evaporator (45°C; until completely dry).

4.10. Add **LC-LOAD** to **MTP PLATE**. Aim for 1 g/L concentration (e.g. 100 µL to 100 µg protein starting material).

4.11. Sonicate **MTP PLATE** tube in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min). *SP*

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 and optimized procedures for chemical labeling: www.preomics.com/faq.

NOTE2 During the digestion, place the silicon mat lightly on top of the **CARTRIDGE**.
Do not close the silicon mat tightly to prevent pressure buildup.

SP - Storage Point: At this point, close the peptide containing tube or **CARTRIDGE** using the silicon mat.
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
ALKYKATION	Specific cysteine modification	$C_6H_{11}NO$	[C]	+113.084Da

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