



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 kit contains everything to perform a complete sample preparation. 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	●	24x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND-BCT	●	4x 2 mL				●	Reconstitutes lyophilized proteolytic enzymes.	RT
LYSE-BCT	●	12x 1 mL			●		Denatures, reduces and alkylates proteins.	RT
STOP	●	12x 1 mL	●	●		●	Stops the enzymatic activity.	RT
WASH 1	●	12x 2 mL	●	●		●	Cleans peptides from hydrophobic contaminants.	RT
WASH 2	●	12x 2 mL		●		●	Cleans peptides from hydrophilic contaminants.	RT
ELUTE	●	12x 2 mL	●		●	●	Elutes the peptides from the cartridge.	RT
LC-LOAD	○	12x 1 mL		●		●	Loads peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		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	Biological fluids & therapeutic proteins. 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.

Procedure



Method

1 LYSE

- 1.1. Add 50 µL **LYSE-BCT** (brown circle) to 1-100 µg of protein sample, place it in a HEATING BLOCK (95°C; 1,000 rpm; 10 min).
Precipitation may occur. ***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).
- 1.4. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of **WASTE PLATE**. Label plate and wells.
- 1.5. Transfer sample to **CARTRIDGE** and cool down (RT). Be careful not to damage the bottom layer of **CARTRIDGE**.

2. DIGEST

- 2.1. Add 210 µL **RESUSPEND-BCT** (yellow circle) to **DIGEST** (red circle) (1 tube for 4 reactions), shake (RT; 500 rpm; 10 min), pipette up/down.
NOTE2
- 2.2. Add 50 µL **DIGEST** (red circle) to **CARTRIDGE** and place it in a pre-heated HEATING BLOCK (37°C; 500 rpm; 1 hour). ***NOTE3***
- 2.3. Add 100 µL **STOP** (black circle) to **CARTRIDGE** (precipitation may occur), shake (RT; 500 rpm; 1 min / pipette up/down). ***SP***

3. PURIFY

- 3.1. Spin **CARTRIDGE** in a CENTRIFUGE (3,800 rcf; 1 min). If needed, adjust time to ensure complete flow-through.
- 3.2. Add 200 µL **WASH 1** (blue circle) to **CARTRIDGE**, repeat step 3.1.
- 3.3. Add 200 µL **WASH 2** (green circle) to **CARTRIDGE**, repeat step 3.1. ***SP***
- 3.4. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of the **MTP PLATE**. Label plate and wells.
- 3.5. Add 100 µL **ELUTE** (pink circle) to **CARTRIDGE**, repeat step 3.1., keep flow-through in **MTP PLATE**.
- 3.6. Repeat step 3.5., keep flow-through in the same **MTP PLATE**.
- 3.7. Discard **CARTRIDGE** and place **MTP PLATE** in a vacuum evaporator (RT; until completely dry but not overnight).
- 3.8. Add **LC-LOAD** (white circle) to **MTP PLATE**. Aim for 1 g/L concentration (e.g. 100 µL to 100 µg protein starting material).
- 3.9. Sonicate **MTP PLATE** 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.

For even lower oxidation & deamidation rates and increased alkylation rate: reduce heating block temperature to 80°C and incubate samples for 10-20 min.

Visit our FAQ website for more information: www.preomics.com/faq.

NOTE2 For optimal digestion efficiency, do not store **DIGEST** (red circle) resuspended in **RESUSPEND-BCT** (yellow circle), but use within one day.

NOTE3 During the digestion, place the provided 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	UNIMOD #
ALKYLATION	Carbamidomethyl on cysteine	C ₂ H ₃ NO	[C]	+57Da	4

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