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PREOMICS

iST Sample Preparation Kit **8x**

Mammalian Tissue

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 bioassay@bioinnovations.in

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		2x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND		1x 2 mL				•	Reconstitutes lyophilized proteolytic enzymes.	RT
LYSE		1x 1 mL			•		Denatures, reduces and alkylates proteins.	RT
STOP		1x 1 mL	•	•		•	Stops the enzymatic activity.	RT
WASH 1		1x 2 mL	•	•		•	Cleans peptides from hydrophobic contaminants.	RT
WASH 2		1x 2 mL		•		•	Cleans peptides from hydrophilic contaminants.	RT
ELUTE		1x 2 mL	•		•	•	Elutes the peptides from the cartridge.	RT
LC-LOAD		1x 1 mL		•		•	Loads peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		8x					Cartridge for 1 to 100 µg protein starting material.	RT
WASTE		8x					2.0 mL tube for collecting waste after washing steps.	RT
COLLECTION		8x					1.5 mL tube for collecting peptides after elution.	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	1-3 mm^3 of mammalian tissue samples (for harder tissues like heart or muscle, use ~1 mm^3).
GLASS BEADS	Protein extraction glass beads (Diagenode #C20000021; Ø<1 mm) to facilitate tissue lysis.
HEATING BLOCK	Two heating blocks are recommended to support protein denaturation and digestion.
CENTRIFUGE	1.5/2.0 mL reaction tube 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

1. LYSE — Reduce & Alkylate — 10 min, 95°C
2. DIGEST — LysC & Trypsin — 180 min, 37°C
3. PURIFY — Wash & Elute — 60 min, RT

Method

1. LYSE

1.1. Place tissue piece in a clean 1.5 mL microreaction LoBind tube. Add 40-50 mg glass beads to sample. *NOTE1*

1.2. Add 100 µL **LYSE**. Shear sample in a SONICATOR (10 cycles; 30 sec ON/OFF). *NOTE2*

1.3. Place sample in a HEATING BLOCK (95°C; 1,000 rpm; 10 min).

1.3. Optional: Spin down droplets (RT; max. 300 rcf; 10 sec).

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 tube and place it in a pre-heated HEATING BLOCK (37°C; 500 rpm; 3 hours).

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

2.4. Spin sample in CENTRIFUGE (16,000 rcf; 1 min).

3. PURIFY

3.1. Use **ADAPTER** to place **CARTRIDGE** in **WASTE** tube. Label all tubes.

3.2. Transfer supernatant from 2.4. to **CARTRIDGE**. Be careful not to damage the bottom layer of **CARTRIDGE**.

3.3. Spin **CARTRIDGE** in a CENTRIFUGE (3,800 rcf; 1-3 min). If needed, adjust time to ensure complete flow-through.

3.4. Add 200 µL **WASH 1** to **CARTRIDGE**, repeat step 3.3.

3.5. Add 200 µL **WASH 2** to **CARTRIDGE**, repeat step 3.3. *SP*

3.6. Use **ADAPTER** to place **CARTRIDGE** in a fresh **COLLECTION** tube. Label all tubes.

3.7. Add 100 µL **ELUTE** to **CARTRIDGE**, repeat step 3.3, keep flow-through in **COLLECTION** tube.

3.8. Repeat step 3.7, keep flow-through in the same **COLLECTION** tube.

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

3.10. Add **LC-LOAD** to **COLLECTION** tube. Aim for 1 g/L concentration (e.g. 100 µL to 100 µg protein starting material).

3.11. Sonicate **COLLECTION** tube in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min). *SP*

NOTE1 1 mg tissue corresponds to ~20-100 µg protein, strongly depending on the tissue type.
Visit our FAQ website for more information on tissue starting amounts: www.preomics.com/faq.

NOTE 2 For harder tissue like heart or muscle, repeat steps 1.3.-1.4. once (sonication > boiling > sonication > boiling).

SP - Storage Point: At this point, close the peptide containing tube or **CARTRIDGE** using silicon lid.
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 #
ALKYKATION	Carbamidomethyl on cysteine	C_2H_3NO	[C]	+57Da	4

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