iST Sample Preparation Kit (8 reactions)
PROTOCOL - Urine

INTRODUCTION

Sample preparation is one of the essential steps of bottom-up proteomics. The PreOmics sample preparation kit is designed to assist you achieving best results with few sample preparation steps and little hands-on time. For sample-specific protocols and optimization contact us or visit our web-site at www.preomics.com.

KIT CONTENTS

The kit contains all you need 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 cleanup.

Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Volatile		
DIGEST	red	2x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	yellow	1x 2 mL				✓	Protease reconstitution buffer for enzymes.	RT
LYSE	brown	1x 1 mL			✓		Denatures, reduces and alkylates proteins.	RT
STOP	black	1x 1 mL	✓	✓		✓	Stops the enzymatic activity.	RT
WASH 0	no color	1x 2 mL	✓	✓		✓	Clean up peptides from tetrapyrolle contaminants.	RT
WASH 1	blue	1x 2 mL	✓	✓		✓	Clean up peptides from hydrophobic contaminants.	RT
WASH 2	green	1x 2 mL		✓		✓	Clean up peptides from hydrophilic contaminants.	RT
ELUTE	violet	1x 2 mL	✓		✓	✓	Elutes the peptides from the cartridge.	RT
LC-LOAD	white	1x 1 mL		✓		✓	Load peptides on reversed-phase LC-MS column.	RT

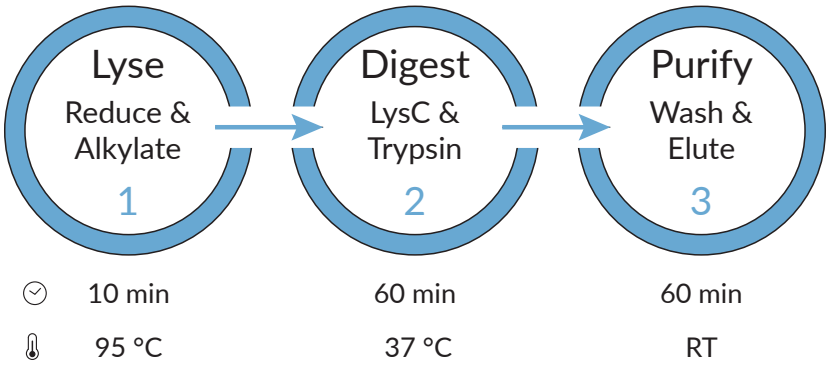
CARTRIDGES	8x	Cartridge for 1 to 100 µg protein starting material.	RT
WASTE	8x	Tube for collecting waste after washing steps.	RT
COLLECTION	8x	Tube for collecting peptides after elution.	RT
ADAPTER	8x	Enables a cartridge to be placed into a tube.	RT
CAP	8x	Cap to (optionally) close the cartridge`s bottom.	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	100 mL urine.
CENTRIFUGAL FILTER	3kDa protein concentration ultrafiltration system (e.g. Merck Millipore ACS500302).
HEATING BLOCK	Two heating blocks are recommended to support protein denaturation and digestion.
CENTRIFUGE	Eppendorf tube centrifuges are necessary for loading, washing and elution.
SONICATOR	If you have DNA in your sample, shear it by sonication (e.g. Diagenode Bioruptor®).
SPEED-VAC	Vacuum manifolds evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

PROCEDURE



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PROTOCOL

1. PREPARE AND LYSE

1.1. Load 10 mL urine onto a centrifugal filter (not provided) and concentrate to 1 mL. Repeat until 100 mL are loaded.

1.2. Concentrate the sample to 100 µL and add 900 µL LYSE ● to your sample. Mix gently.

1.3. Concentrate the solution to 50 µL, mix thoroughly and transfer the sample to a fresh tube.

1.4. Place the sample in a HEATING BLOCK (95°C; 1,000 rpm; 10 min). *NOTE 1*

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

1.6. If you expect DNA, shear sample in a SONICATOR (10 cycles; 30 sec ON/OFF).

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

1.8. 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** ○ to **DIGEST** ● (1 tube for 4 reactions), shake (RT; 500 rpm; 10 min), pipette up/down.

2.2. Add 50 µL **DIGEST** ● to **CARTRIDGE** and place it in a pre-heated HEATING BLOCK (37 °C; 500 rpm; 1-3 hours).

2.3. Add 100 µL **STOP** ● 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; 1-3 min). If needed, adjust values to ensure complete flow-through.

3.2. Load 200 µL WASH 0 (no color), repeat step 3.1.

3.3. Add 200 µL **WASH 1** ● to **CARTRIDGE**, repeat step 3.1.

3.4. Add 200 µL **WASH 2** ● to **CARTRIDGE**, repeat step 3.1. *SP*

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

3.6. Add 100 µL **ELUTE** ● to **CARTRIDGE**, repeat step 3.1., keep flow-through in **COLLECTION** tube.

3.7. Repeat step 3.6., keep flow-through in the same **COLLECTION** tube.

3.8. Discard **CARTRIDGE** and place **COLLECTION** tube in a SPEED-VAC (45 °C; until completely dry).

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

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

NOTE 1 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: www.preomics.com/faq.

SP - Storage Point: At this point, close the peptide containing tube or **CARTRIDGE** (use a **CAP** for bottom).

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_2H_3NO	[C]	+57 Da	4

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