Sample Preparation Kit
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 website 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 digest and a final clean up.

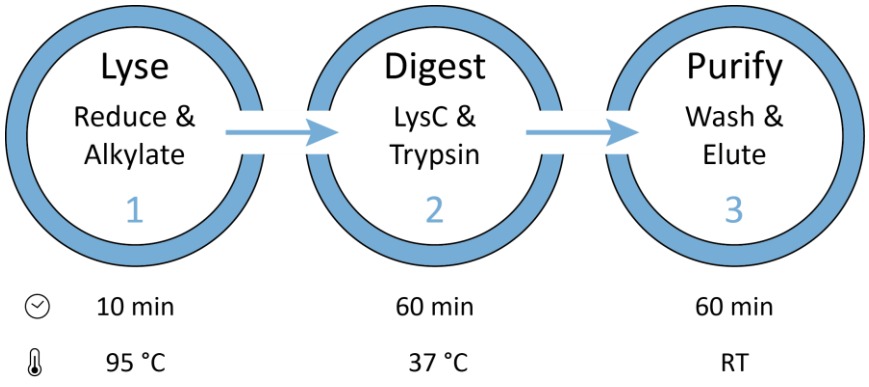
COMPONENT	CAP	QUANTITY	BUFFER PROPERTIES Organic	Acidic	Basic	Volatile	DESCRIPTION	STORAGE
DIGEST	red	2x					Enzyme *Trypsin*-mix to digest proteins.	-20 °C
RESUSPEND	yellow	1x 1 mL				✓	Protease reconstitution buffer for enzymes.	RT
LYSE	brown	4x 2 mL			✓		Denature, reduce and alkylate proteins.	RT
STOP	black	1x 1 mL	✓	✓		✓	Stop the enzymatic activity.	RT
WASH 0	no color	1x 2mL	✓	✓		✓	Clean up peptides.	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	✓		✓	✓	Elute the peptides from the cartridge.	RT
LC-LOAD	white	1x 1 mL		✓		✓	Load peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		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 placing a cartridge 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	Heating shakers are recommended to help protein denaturation and during 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.

PROCEDURE



Sample Preparation Kit
PROTOCOL – Urine

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 pre-heated HEATING BLOCK (95 °C; 1,000 rpm; 10 min).

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

1.6. Transfer sample to **CARTRIDGE** and cool down (RT). Be careful not to damage the bottom layer of **CARTRIDGE**.

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

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 happen), 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., discard flow-through.

3.3. Add 200 µL **WASH 1** ● to **CARTRIDGE**, repeat step 3.1., discard flow-through.

3.4. Add 200 µL **WASH 2** ● to **CARTRIDGE**, repeat step 3.1., discard flow-through. *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. Remove **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 a SONICATOR (10 cycles 30 sec ON/OFF). *SP*

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 2 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

DISCLAIMER