PREOMICS

iST Fractionation Add-on

Washed peptides

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 peptide fractionation into three fractions.

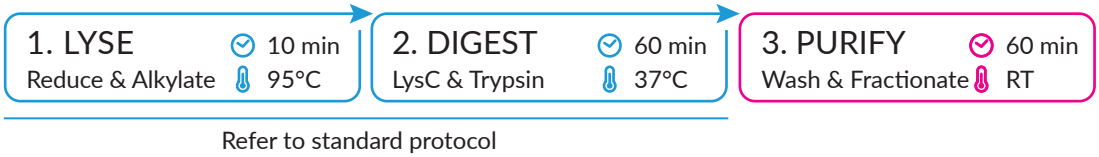
Component	Cap	Quantity			Buffer Properties				Description	Storage
		8rxn	12rxn	96rxn	Organic	Acidic	Basic	Volatile		
FRACTION-1		1x 2 mL	2x 2 mL	12x 2 mL	●		●	●	Fractionates the peptides from the cartridge, fraction 1.	RT
FRACTION-2		1x 2 mL	2x 2 mL	12x 2 mL	●		●	●	Fractionates the peptides from the cartridge, fraction 2.	RT
FRACTION-3		1x 2 mL	2x 2 mL	12x 2 mL	●		●	●	Fractionates the peptides from the cartridge, fraction 3.	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	Washed peptides bound to the cartridge.
CENTRIFUGE	1.5/2.0 mL reaction tube centrifuges are required for elution.
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. Refer to standard protocol

2. DIGEST

2.1. Refer to standard protocol

3. PURIFY

3.1. to 3.4. Refer to standard iST protocol *NOTE 1*

3.5. Add 200 µL **FRACTION-1** ● to **CARTRIDGE**, spin in CENTRIFUGE (1,000 rcf, 1 min).
Keep flow-through in **COLLECTION** tube 1. Transfer **CARTRIDGE** to a new **COLLECTION** tube.

3.6. Add 200 µL **FRACTION-2** ● to **CARTRIDGE**, spin in CENTRIFUGE (1,000 rcf, 1 min).
Keep flow-through in **COLLECTION** tube 2. Transfer **CARTRIDGE** to a new **COLLECTION** tube.

3.7. Add 200 µL **FRACTION-3** ● to **CARTRIDGE**, spin in CENTRIFUGE (1,000 rcf, 1 min).
Keep flow-through in **COLLECTION** tube 3.

3.8. Discard **CARTRIDGE** and place **COLLECTION** tubes 1-3 in a vacuum evaporator (45°C; until completely dry).

3.9. Add **LC-LOAD** ○ to **COLLECTION** tubes 1-3. Aim for 1 g/L concentration remembering that the starting protein concentration will have been fractionated into 3 (e.g. 90 µL to 90 µg protein starting material equates to 30 µL per tube). *NOTE 2*

3.10. Sonicate **COLLECTION** tubes 1-3 in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min). *SP*

Note 1: For iST-NHS, refer to the protocol up to 4.6.

Note 2: Because of the nature of the buffers, we recommend using BCA assay rather than measuring the peptide concentration with absorption (A260 or A280).

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 fixed modifications as referred to in the corresponding kit protocol

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