iST Sample Preparation High-Throughput Kit (192 rxn)
PROTOCOL - Pelleted cells & precipitated protein

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	2 vials					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	yellow	2x 10 mL				✓	Protease reconstitution buffer for enzymes.	RT
LYSE	brown	2x 10 mL			✓		Denatures, reduces and alkylates proteins.	RT
STOP	black	2x 13 mL	✓	✓		✓	Stops the enzymatic activity.	RT
WASH 1	blue	2x 25 mL	✓	✓		✓	Clean up peptides from hydrophobic contaminants.	RT
WASH 2	green	2x 25 mL		✓		✓	Clean up peptides from hydrophilic contaminants.	RT
ELUTE	violet	2x 25 mL	✓		✓	✓	Elutes the peptides from the cartridge.	RT
LC-LOAD	white	2x 13 mL		✓		✓	Load peptides on reversed-phase LC-MS column.	RT
CARTRIDGES		2x 96					Cartridge for 1 to 100 µg protein starting material.	RT
WASTE PLATE		2x					Deep well plate for collecting waste after washes.	RT
MTP PLATE		2x					LoBind plate for collecting peptides after elution.	RT
ADAPTER PLATE		2x					Enables cartridges to be placed on top of 96w plates.	RT

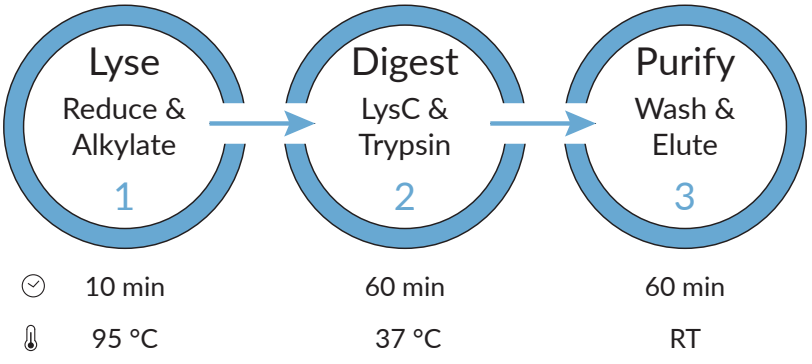
PRE-REQUISITES

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	Pelleted cells or precipitated protein. For other materials ask us for adapted protocols.
96x-WELL PLATES	96 deep well and PCR plates with skirt to balance WASTE & MTP plates in centrifuge.
HEATING BLOCK*	Two MTP plate heaters are recommended to support protein denaturation and digestion.
CENTRIFUGE	Swing-bucket MTP plate 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.

* a heating shaker adapter for MTP plate shakers can be purchased from PreOmics

PROCEDURE



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PROTOCOL

1. LYSE *Critical Note*

1.1. Add 50 µL **LYSE** ● to 1-100 µg of protein sample, place it in a HEATING BLOCK (95°C; 1,000 rpm; 10 min). *NOTE 1*

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

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

1.4. Use **ADAPTER PLATE** to place **CARTRIDGES** on top of the **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 5 mL **RESUSPEND** ○ to **DIGEST** ● (1 vial for 96 reactions), invert vial several times (RT; 10 min). *NOTE2*

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.

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

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

3.4. Use **ADAPTER PLATE** to place **CARTRIDGES** on top of the **MTP PLATE**. Label plate and wells.

3.5. Add 100 µL **ELUTE** ● 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** tube in a SPEED-VAC (45 °C; until completely dry).

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

3.9. Sonicate **MTP PLATE** in a SONICATOR (10 cycles; 30 sec ON/OFF) or shake (RT; 500 rpm; 5 min). *SP*

Critical Note: For automation processes, only use Protein LoBind plates as buffer reservoirs to avoid polymer contamination. Contact us at info@preomics.com for advice on buffer and plastic ware usage on liquid handling platforms.

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.

NOTE 2 Lyophilized enzyme mix is stable for 9 months at -20°C.

Resuspended enzyme mix can be stored for 4 weeks at -20°C with maxmium 5 freeze/thaw cycles.

SP - Storage Point: At this point, peptides can be frozen at -20 °C.

Storage of peptides should not exceed two weeks at -20 °C. For extended storage, store peptides 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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