Sample Preparation Kit
PROTOCOL - CHEMICAL LABELING

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.

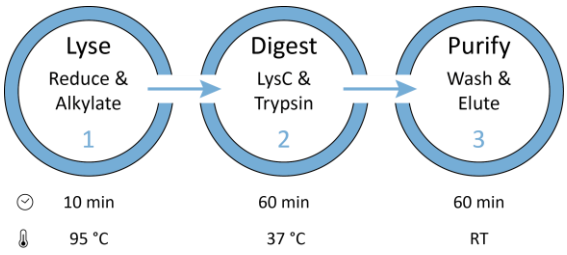
COMPONENT	CAP COLOR	QUANTITY	BUFFER PROPERTIES Organic	Acidic	Basic	Volatile	DESCRIPTION	STORAGE TEMP.
DIGEST	red	2x				✓	Enzyme *Trypsin*-mix to digest proteins.	-20 °C
RESUSPEND	yellow	1x 2 mL				✓	Protease reconstitution buffer for enzymes.	RT
LYSE-NHS	orange	1x 1 mL			✓		Denature, reduce and alkylate proteins.	RT
STOP	black	1x 1 mL	✓	✓		✓	Stop the enzymatic activity.	RT
WASH 1	blue	1x 2 mL	✓	✓		✓	Clean up peptides from hydrophobic materials.	RT
WASH 2	green	1x 2 mL		✓		✓	Clean up peptides from hydrophilic materials.	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 max. 100 µg protein starting material (max. loading volume per cartridge is 300 µL)	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	Lyophilized protein or pelleted cells. For other materials ask us for adapted protocols.
HEATING BLOCK	2x heaters 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.
LABELING REAGENT	Labelling reagent (400 µg labeling reagent in 21 µL dry acetonitrile for 100 µg peptides).
LABELING BUFFERS	Dry acetonitrile & quenching buffer (5% hydroxylamine) as recommended by the manufacturer.

PROCEDURE



DISCLAIMER

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PROTOCOL

1. LYSE

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

1.2. Optional: Spin down droplets (RT; max. 300 rcf; 10 sec). *NOTE 1*

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

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

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

2. DIGEST & LABEL

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 21 µL dry acetonitrile to LABELING REAGENT (see page 1; 4:1 ratio of label:peptides). *NOTE 2*

2.4. Add resuspended LABELING REAGENT to **CARTRIDGE**, incubate shaking (RT; 1 hour).

2.5. Add 10 µL QUENCHING BUFFER (5% Hydroxylamine) to **CARTRIDGE** and mix.

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

3.3. Add 200 µL **WASH 2** ● to **CARTRIDGE**, repeat step 3.1., discard flow-through. *SP*

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

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

3.6. Repeat step 3.5., keep flow-through in the same **COLLECTION** tube.

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

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

3.9. Sonicate **COLLECTION** tube in a SONICATOR (10 cycles 30 sec ON/OFF). *SP*

NOTE 1
Most classical protein quantification assays are compatible with our lysis buffer. We recommend the BCA assay or the Tryptophan quantification method. Some assays require dilution with water to achieve best results (Dilutions: BCA: none; Bradford: 1:4; Coomassie: 1:20; Lowry: 1:4; Tryptophan: none).

NOTE 2 We recommend to use only fresh labeling reagents at a label to peptide ratio of 4:1 to achieve best labeling and identification results. In case of reduced labeling efficiency, use LABELING REAGENT concentrations according to the manufacturer`s instructions and see the manufacturer`s troubleshooting information.

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
ALKYLATION	Specific cysteine modification	$C_6H_{11}NO$	[C]	+113.084 Da