iST-NHS Sample Preparation Kit (96 reactions)
PROTOCOL - CHEMICAL LABELING

INTRODUCTION

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

KIT CONTENTS

The iST-NHS kit provides a streamlined solution for reliable sample preparation compatible with chemical labeling such as iTRAQ or TMT. 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	24x				●	Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	yellow	4x 2 mL				●	Protease reconstituion buffer for enzymes.	RT
LYSE-NHS	orange	12x 2 mL			●		Denatures, reduces and alkylates proteins.	RT
STOP	black	12x 1 mL	●	●		●	Stops the enzymatic activity.	RT
WASH 1	blue	12x 2 mL	●	●		●	Clean up peptides from hydrophobic contaminants.	RT
WASH 2	green	12x 2 mL		●		●	Clean up peptides from hydrophilic contaminants.	RT
ELUTE	violet	12x 2 mL	●		●	●	Elute the peptides from the cartridge.	RT
LC-LOAD	white	12x 1 mL		●		●	Load peptides on reversed-phase LC-MS column.	RT

CARTRIDGES	96x	Cartridge for 1 to 100 µg protein starting material.	RT
WASTE PLATE	1x	Deep well plate for collecting waste after washes.	RT
MTP PLATE	1x	LoBind plate for collecting peptides after elution.	RT
ADAPTER PLATE	1x	Enables cartridges to be placed on top of 96w plates.	RT
ADAPTER	8x	Enables a cartridge to be placed into a tube.	RT
CAP	96x	Cap to (optionally) close the cartridge`s bottom.	RT

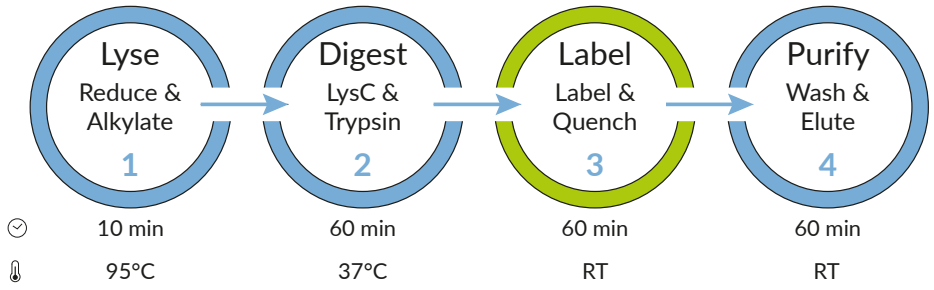
PRE-REQUISITES

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	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.
LABELING REAGENT	Labeling reagent (e.g 400 µg labeling reagent in 41 µL dry acetonitrile for 100 µg peptides).
LABELING BUFFER	Dry acetonitrile & quenching buffer (5% hydroxylamine), as recommended by the manufacturer.

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

PROCEDURE



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PROTOCOL

1. LYSE

1.1. Add 50 µL **LYSE-NHS** ● to 1-100 µg of protein sample, place it in a 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).

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 **sample** and place it in a pre-heated HEATING BLOCK (37 °C; 500 rpm; 1-3 hours).

2.3. Resuspend LABELING REAGENT in dry acetonitrile (see page 1; 4:1 ratio of label:peptides). *NOTE 2*

2.4. Add resuspended LABELING REAGENT to **sample**, pipette up/down, incubate shaking (RT; 500 rpm; 1 hour).

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

2.6. Add 100 µL **STOP** ● to **sample** (precipitation may occur), shake (RT; 500 rpm; 1 min), pipette up/down. *SP*

3. PURIFY

3.1. Use **ADAPTER PLATE** to place **CARTRIDGES** on top of the **WASTE PLATE** tube. Label plate and wells.

3.2 Transfer **sample** to **CARTRIDGE**. Be careful not to damage the bottom layer of **CARTRIDGE**.

3.3 Spin **CARTRIDGE** in a CENTRIFUGE (3,800 rcf; 1-3 min). If needed, adjust values to ensure complete flow-through.

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

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

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** in a SPEED-VAC (45 °C; until completely dry).

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

3.9. Sonicate **MTP PLATE** 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.

Most classical protein quantification assays are compatible with our lysis buffer. We recommend the BCA assay or 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).

Visit our FAQ website for more information: www.preomics.com/faq.

NOTE 2 We recommend to use only fresh labeling reagents at a label to peptide ratio of 4:1, as well as a min. 30% (v/v) acetonitrile concentration during the labeling reaction 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 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
ALKYLATION	Specific cysteine modification	$C_6H_{11}NO$	[C]	+113.084 Da

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