# PREOMICS

# Phoenix 96x

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## Peptide samples

## Introduction

Detergent-, salt-, and contaminant-free peptide samples are essential for bottom-up proteomics. The PreOmics® Phoenix Peptide Cleanup 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/resources or contact info@preomics.com.

## Kit Contents

The kit contains all chemicals and plasticware to perform an efficient peptide cleanup, removing detergents, fatty acids, sugars, salts, and other contaminants.

| Component | Cap | Quantity | Buffer Properties | | | Description | Storage |
|---|---|---|---|---|---|---|---|
| | | | Organic | Acidic | Basic | | |
| STOP | ● | 1x 15 mL | ● | ● | | For sample acidification to promote efficient peptide binding. | RT |
| WASH X | ● | 3x 25 mL | ● | ● | | For removing hydrophobic contaminants. | RT |
| WASH 1 | ● | 2x 25 mL | ● | ● | | For removing hydrophobic contaminants. | RT |
| WASH 2 | ● | 1x 25 mL | | ● | | For removing hydrophilic contaminants. | RT |
| ELUTE | ● | 1x 25 mL | ● | | ● | For eluting peptides from the cartridge. | RT |
| LC-LOAD | ● | 1x 25 mL | | ● | | For loading peptides on reversed-phase LC-MS column. | RT |
| CARTRIDGE | | 96x | | | | Cartridges with SPE sorbent for peptide purification from 1–100 µg protein starting material. Racked in adapter plate and sealed with silicone mat. | RT |
| WASTE PLATE | | 1x | | | | Deep well plate for collecting waste after washes. | RT |
| COLLECTION PLATE | | 1x | | | | LoBind® plate for collecting peptides after elution. The plate has a max. working volume of 150 µL, and can be used after validating the workflow for elution with lower buffer volumes. Alternatively, use ELUTE PLATE (see Pre-Requisites section below). | RT |
| ADAPTER PLATE | | 1x | | | | Enables cartridges to be placed on top of 96-well plates. | RT |
| ADAPTER | | 8x | | | | Enables a cartridge to be placed into a tube. | 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 | Peptide solution (20–100 µL) generated from tryptic digest of 1–100 µg protein starting material. For other sample types, contact PreOmics for adapted protocols. |
| 96 WELL PLATES | 96 deep well & 96 well skirted plates to balance WASTE & COLLECTION PLATES in centrifuge. |
| CENTRIFUGE | Swing-bucket centrifuges are required for peptide loading, washing, and elution. |
| ELUTE PLATE | 96 deep well plate with >250 µL for elution of peptides from cartridge (e.g., Eppendorf Deepwell plate 96/500 µL Protein LoBind®, catalogue number EU: 0030504100, US: 951032107). |
| SEALING MAT | Prevents sample contamination and evaporation (e.g., Eppendorf Sealing Mat, catalogue number EU&US: 0030127978). |
| VACUUM EVAPORATOR | To evaporate volatile buffers from the eluate before LC-MS. |
| ULTRASONIC BATH | Optional: can be used to resuspend peptides. |

## Procedure


## Method

### 1. LOAD

1.1. Mix 20–100 µL SAMPLE with equal volume of **STOP** ●. *NOTE1*

1.2. Place **ADAPTER PLATE** with CARTRIDGE on **WASTE PLATE**. Label plate and wells, and transfer the sample to the **CARTRIDGE**. *NOTE2*

### 2. PURIFY

2.1. Spin **CARTRIDGE** in a CENTRIFUGE (2,250 rcf; 1–3 min). If needed, adjust time to ensure complete flow-through.

2.2. Add 200 µL **WASH X** ○ to **CARTRIDGE**, repeat step 2.1, discard flow-through.

2.3. Repeat step 2.2 twice.

2.4. Add 200 µL **WASH 1** ● to **CARTRIDGE**, repeat step 2.1, discard flow-through.

2.5. Repeat step 2.4 once.

2.6. Add 200 µL **WASH 2** ● to **CARTRIDGE**, repeat step 2.1, discard flow-through. *SP*

## 3. ELUTE

3.1. Discard **WASTE PLATE**. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of the ELUTE PLATE. Label plate and wells.
Alternatively, use the supplied **COLLECTION PLATE** after validating the workflow for elution with lower buffer volumes. See “Kit Contents” for details.

3.2. Add 100 µL **ELUTE** ● to **CARTRIDGE**, spin CARTRIDGE in a CENTRIFUGE (2,250 rcf; 1–3 min).
Keep flow-through in ELUTE PLATE.

3.3. Repeat step 3.2, keep flow-through in the same ELUTE PLATE.

3.4. Discard **CARTRIDGE** and place and place ELUTE PLATE in a VACUUM EVAPORATOR (45°C; until completely dry). *SP*

3.5. Reconstitute peptides by adding **LC-LOAD** ○ to ELUTE PLATE. Adjust the volume according to specific requirements.
For example, add 50 µL **LC-LOAD** to 100 µg protein starting material.

3.6. Sonicate ELUTE PLATE in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min).

3.7. Spin plate in a CENTRIFUGE as follows:
- User-provided ELUTE PLATE: RT; maximum rcf recommended by manufacturer; 5–15 min.
- COLLECTION PLATE: RT; 2,250 rcf; 15 min.

Transfer the supernatant to a clean plate and avoid touching the bottom of the well during transfer.*NOTE3*

| | |
|---|---|
| *NOTE1* | The SAMPLE-STOP mix is expected to be acidic (pH<3.0) when the peptide sample was stored in a buffer commonly used for tryptic digestion. If the pH is above 3.0, contact info@preomics.com for an optimized protocol. The pH of the mixture can be measured with pH paper (the STOP pH itself cannot be determined using a pH measurement device or pH paper due to the high concentration of organic solvent). |
| *NOTE2* | Alternatively, use **ADAPTERS** to place single **CARTRIDGES** on top of 2 mL tubes. Refer to the corresponding Phoenix 4x kit protocol for subsequent steps. |
| *NOTE3* | At this point, peptide concentration can be measured, or the sample can be directly injected for LC-MS analysis. Visit our FAQ for recommendations on peptide quantitation assays. The silicone mat provided with the kit is incompatible with autosamplers. |
| *SP* - Storage Point: | At this point, close the peptide containing plate or **CARTRIDGE** using the silicone mat. Peptides can be frozen at -20°C for two weeks. Dried peptides, prior to reconstitution in LC-LOAD, can also be stored long-term at -80°C. |

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