

Phoenix Peptide Cleanup Kit (96 reactions) PROTOCOL

INTRODUCTION

Detergent-, salt- and contaminant-free peptide samples are essential for bottom-up proteomics. The PreOmics Phoenix Peptide Cleanup 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 an efficient peptide cleanup removing detergents, fatty acids, sugars, salts and other contaminants. It includes all chemicals to perform a final cleanup of your peptides.

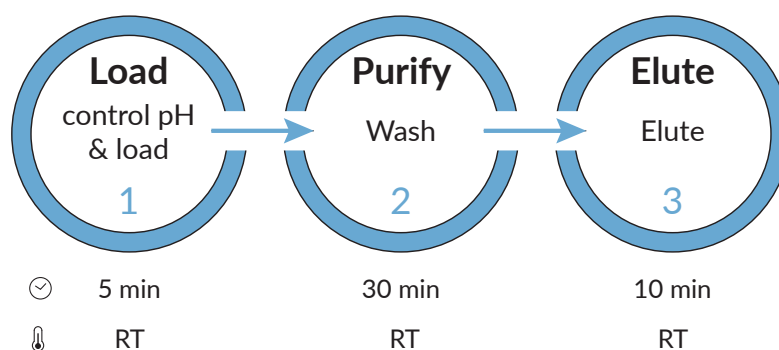
| Component | Cap | Quantity | Buffer Properties | | | | Description | Storage |
|---------------|---------|----------|-------------------|--------|-------|----------|---|---------|
| | | | Organic | Acidic | Basic | Volatile | | |
| STOP | black | 1x 25 mL | ☞ | ☞ | | ☞ | Acidify sample for efficient peptide binding. | RT |
| WASH X | crystal | 3x 25 mL | ☞ | ☞ | | ☞ | Clean up peptides from hydrophobic contaminants. | RT |
| WASH 1 | blue | 2x 25 mL | ☞ | ☞ | | ☞ | Clean up peptides from hydrophobic contaminants. | RT |
| WASH 2 | green | 1x 25 mL | | ☞ | | ☞ | Clean up peptides from hydrophilic contaminants. | RT |
| ELUTE | violet | 1x 25 mL | ☞ | | ☞ | ☞ | Elute the peptides from the cartridge. | RT |
| LC-LOAD | white | 1x 12 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 single 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 | Peptide solution. |
| 96x-WELL PLATES | 96 deep well and PCR plates with skirt to balance WASTE & MTP plates in centrifuge. |
| CENTRIFUGE | Swing-bucket MTP plate centrifuges are necessary for loading, washing and elution. |
| SPEED-VAC | Vacuum manifolds evaporate volatile buffers from the eluate before LC-MS. |
| ULTRASONIC BATH | Optional: can be used to resuspend peptides. |

PROCEDURE



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PROTOCOL

1. LOAD

- 1.1. Control the pH of your peptide sample, it should be acidic (pH<3.0). If it is too basic, acidify with **STOP ●**. ***NOTE***
- 1.2. Use **ADAPTER PLATE** to place **CARTRIDGES** on top of the **WASTE PLATE**. Label plate and wells.
- 1.3. Transfer sample to **CARTRIDGE**. Be careful not to damage the bottom layer of **CARTRIDGE**.

2. PURIFY

- 2.1. Spin **CARTRIDGE** in a CENTRIFUGE (3,800 rcf; 1-3 min). If needed, adjust values 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. Use **ADAPTER PLATE** to place **CARTRIDGES** on top of the **MTP PLATE**. Label plate and wells.
- 3.2. Add 100 µL **ELUTE ●** to **CARTRIDGE**, spin **CARTRIDGE** in a CENTRIFUGE (3,800 rcf; 1-3 min).
Keep flow-through in **MTP PLATE**.
- 3.3. Repeat step 3.2, keep flow-through in the same **MTP PLATE**.
- 3.4. Discard **CARTRIDGE** and place **MTP PLATE** tube in a SPEED-VAC (45 °C; until completely dry).
- 3.5. Add **LC-LOAD ○** to **MTP PLATE** tube. Aim for 1 g/L concentration (e.g. 100 µL to 100 µg protein starting material).
- 3.6. Sonicate **MTP PLATE** in a SONICATOR (10 cycles; 30 sec ON/OFF) or shake (RT; 500 rpm; 5 min). ***SP***

***NOTE *** To avoid losing peptides, you may control the pH by testing the buffer in which the peptides are stored in. You may dilute your sample 1:1 with **STOP**. The maximum loading volume of the **CARTRIDGE** is 200 µL.

***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.

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