

# PHOENIX Peptide Cleanup Kit PROTOCOL



## INTRODUCTION

Detergent-, salt- and contaminant-free peptide samples are essential for bottom-up proteomics. The PreOmics 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](http://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 and prepare them for LC-MS.

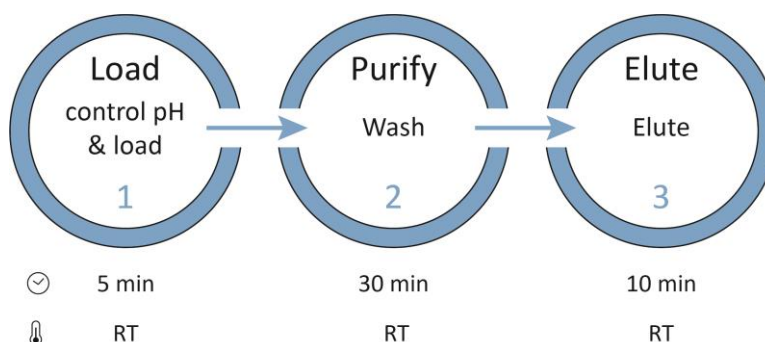
COMPONENT	CAP	QUANTITY	BUFFER PROPERTIES				DESCRIPTION	STORAGE
			Organic	Acidic	Basic	Volatile		
STOP	black	1x 15 mL	✓	✓		✓	Acidify sample for efficient peptide binding.	RT
WASH X	crystal	3x 25 mL	✓	✓		✓	Clean up peptides from hydrophobic contaminants.	RT
WASH 1	blue	2x 25 mL	✓	✓		✓	Clean up peptides 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					Cartridges 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 placing cartridges in the 96well MTP plate.	RT
ADAPTERS		8x					Enables placing a cartridge into a single tube.	RT
CAP		96x					Caps 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	Your peptide solution.
CENTRIFUGE	Eppendorf tube centrifuges are necessary for loading, washing and elution.
SPEED-VAC	Vacuum manifolds evaporate volatile buffers from the eluate before LC-MS.

## PROCEDURE



# PHOENIX Peptide Cleanup Kit PROTOCOL



## 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. Transfer sample to **CARTRIDGE**. Be careful not to damage the bottom layer of **CARTRIDGE**.
- 1.3. Use **ADAPTER PLATE** to place **CARTRIDGES** on top of **WASTE PLATE**. Label all plates and wells.

### 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.
- 2.3. Add 200 µL **WASH 1** ● to **CARTRIDGE**, repeat step 2.1., discard flow-through.
- 2.4. Repeat step 2.3 once.
- 2.5. 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 **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. Remove **CARTRIDGES** and place **MTP PLATE** in a SPEED-VAC (45 °C; until completely dry).
- 3.5. Add **LC-LOAD** ○ to **MTP PLATE**. 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). *\*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 2 weeks at -20 °C. For extended storage, finish the protocol and store at -80 °C.

## DISCLAIMER

Products are warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation"). No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to three months from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample. NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE GRANTED, INCLUDING WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR ANY PARTICULAR PURPOSE, OR NON INFRINGEMENT. BUYER'S EXCLUSIVE REMEDY FOR NONCONFORMING PRODUCTS DURING THE WARRANTY PERIOD IS LIMITED TO REPAIR, REPLACEMENT OF OR REFUND FOR THE NONCONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR PRODUCTS AS THE RESULT OF (I) ACCIDENT, DISASTER OR EVENT OF FORCE MAJEURE, (II) MISUSE, FAULT OR NEGLIGENCE OF OR BY BUYER, (III) USE OF THE PRODUCTS IN A MANNER FOR WHICH THEY WERE NOT DESIGNED, OR (IV) IMPROPER STORAGE AND HANDLING OF THE PRODUCTS. Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.