PREOMICS

Phoenix Peptide Cleanup Kit **96x**

Peptide samples

Peptide Cleanup Solutions
Phoenix
www.preomics.com

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/downloads 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	Volatile		
STOP	●	1x 15 mL	●	●		●	Acidifies sample for efficient peptide binding.	RT
WASH X	●	3x 25 mL	●	●		●	Cleans peptides from hydrophobic contaminant.	RT
WASH 1	●	2x 25 mL	●	●		●	Cleans peptides from hydrophobic contaminants.	RT
WASH 2	●	1x 25 mL		●		●	Cleans peptides from hydrophilic contaminants.	RT
ELUTE	●	1x 25 mL	●		●	●	Elutes the peptides from the cartridge.	RT
LC-LOAD	●	1x 25 mL		●		●	Loads peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		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

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 sample types contact PreOmics for adapted protocols.
96 WELL PLATES	96 deep well & 96 well skirted plates to balance WASTE & MTP PLATES in centrifuge.
CENTRIFUGE	Swing-bucket centrifuges are required for loading, washing and elution.
SONICATOR	If the sample contains DNA, shear it by sonication (e.g. Diagenode Bioruptor®).
VACUUM EVAPORATOR	Vacuum manifolds evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

Procedure

1. LOAD — control pH & load — 5 min — RT
2. PURIFY — Sample wash — 30 min — RT
3. ELUTE — Sample elution — 10 min — RT

Method

1. LOAD

1.1. Control the pH of your peptide sample, it should be acidic (pH<3.0). If the pH is too basic, acidify with **STOP** ●. *NOTE1*

1.2. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of **WASTE PLATE** tube. 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 (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. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of the **MTP PLATE**. Label plate and wells.

3.2. Add 100 µL **ELUTE** ● to **CARTRIDGE**, spin CARTRIDGE in a CENTRIFUGE (2,250 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** in a vacuum evaporator (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 an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min). *SP*

NOTE1 To avoid loosing peptides, control the pH by testing the buffer in which the peptides are stored in.
If the pH is too basic, dilute 1:1 with **STOP**. The maximum loading volume of the **CARTRIDGE** is 200 µL.
Visit our FAQ website for more information: www.preomics.com/faq.

SP -

Storage Point: At this point, close the peptide containing tube or **CARTRIDGE** using silicon mat.
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.

Please refer to www.preomics.com for our General Terms and Conditions.