Application Note
Phoenix Peptide Cleanup Kit

OBJECTIVE

The PreOmics Phoenix Kit offers a simple-to-use solution to clean up peptide mixtures and removes detergents, polymers, salts, lipids and more for reliable LC-MS measurements.

BACKGROUND

Detergents are amphipathic molecules containing both a polar head group and a hydrophobic chain. They are essential for proteomic sample preparation in order to efficiently solubilize proteins, in particular for hydrophobic membrane proteins. However, high concentrations of detergents can interfere with protein or peptide analysis, such as protease digestion, isoelectric focusing, electrospray ionization or column binding and elution. Therefore, detergent removal after the cell lysis is crucial to improve proteomic experiments and to prevent contamination of mass spectrometers.
In this application note, we employ the Phoenix Peptide Cleanup Kit (P.O.00023, PreOmics GmbH) to determine the removal efficiency of various detergents from peptide solutions and the recovery efficiency of a complex peptide mixture.

MATERIAL AND METHODS

Baker`s yeast (*Saccharomyces cerevisiae*) derived from a yeast cube was resuspended in PBS. Aliquots of OD_{600} = 1 were harvested, centrifuged and the pellets were frozen at -20 °C until use. Peptide solutions from the yeast pellets were prepared with the iST Sample Preparation Kit (P.O.00001, PreOmics GmbH).

Next, the tryptic peptide solutions were mixed with 1% [v/v] of the following detergents:

Sample	Detergent spiked-in
A	CHAPS (cholamidopropyl dimethylammonio propane sulfonate, Sigma #C3023)
B	IGEPAL CA-630 (octylphenoxypolyethoxyethanol, Sigma #I8896)
C	PEG-10k (polyethyleneglycol, Millipore #8218811000)
D	SDC (sodium deoxycholate, Sigma #30970)
E	SDS (sodium dodecyl sulfate, Sigma #74255)
F	Triton X-100 (octyl phenol ethoxylate, Sigma #X100)
G	Tween-20 (polyoxyethylene (20) sorbitan monolaurate, Sigma #P1379)
H	H_2O control (no detergent)

The peptide/detergent mix was then transferred to CARTRIDGES and washed with Phoenix wash buffers according to the protocol of the Phoenix Peptide Cleanup Kit (P.O.00023, Preomics GmbH). MS analysis was performed basically as described (1) on an Orbitrap XL mass spectrometer (Thermo Fisher Scientific). Data analysis was performed using the MaxQuant (2) and Perseus software tools (3).

RESULTS

We determined the removal efficiency of the tested detergents by comparing peptide mixtures loaded to the CARTRIDGES with or without the wash buffers in the PHOENIX kit. After treatment with the Phoenix wash buffers, visual inspection of the samples already showed a substantial clean-up of the otherwise milky peptide solution with 1% detergent (Figure 1A). LC-MS/MS analysis revealed efficient removal of detergents, as exemplified by the absence of polyoxyethylene polymers with the characteristic mass shift of +44 m/z in the samples treated with the Phoenix wash buffers (Figure 1B+C). In addition, ion suppression caused by the interaction of the detergent with the HPLC column is strongly reduced leading to overall higher ion intensities.

Next, we quantified the removal efficiency of all tested detergents and found that the Phoenix kit was able to remove more than 99.5% of the spiked-in detergents from the samples, respectively (Figure 1D). The only exception was Tween-20, for which the Phoenix kit was able to remove >85%, due to the high repeat numbers of polytheylene glycol in Tween-20.

1% detergent [v/v]	Removal efficiency (%)
CHAPS	> 99.5
IGEPAL	> 99.5
PEG-10k	> 99.5
SDC	> 99.5
SDS	> 99.5
Triton X-100	> 99.5
Tween-20	> 85.0

Besides removing detergents efficiently, the sample loss during the cleanup process has to be minimal. Thus, we assessed the peptide and protein recovery of the Phoenix kit. To this end, we prepared a tryptic yeast lysate without detergents and loaded it on our CARTRIDGES, followed by either washing with the three Phoenix wash buffers or H_2O wash controls, followed by LC-MS/MS analysis. When comparing the Phoenix washes to H_2O controls, the number of protein and peptide identifications dropped by 6.6 and 9.0%, respectively; the median protein and peptide intensities only dropped by 0.3 and 2.1%, respectively (Figure 2).

In order to evaluate whether peptide/protein recovery depends on the overall abundance, we compared protein intensities of four randomly selected proteins that span the entire abundance range (Figure 2B). Again, we only observed minor intensity drops of the four selected proteins.

CONCLUSION

Clean peptide samples without detergent contamination greatly increase HPLC column lifetime and prevent mass spectrometry downtime. Here, we demonstrate that the Phoenix Peptide Cleanup Kit removes detergents with exceptional efficiency while keeping high peptide recovery rates.
The Phoenix kit is fully compatible with the iST sample preparation kit and downstream LC-MS/MS analyses.

REFERENCES

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2. S Tyanova, T Temu and J Cox.
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The Perseus computational platform for comprehensive analysis of (prote)omics data. Nat Methods (2016) 13:731-740. doi: 10.1038/nmeth.3901.

PRODUCTS

Product	Quantity	Manufacturer	Product Code	Price
Phoenix Kit 96x	96 reactions	PreOmics	P.O.00023	EUR 1599

ORDERING INFORMATION

USA
http://www.preomics.com/products
order@preomics.com
Tel: +1-908-842-3301

all other countries
http://www.preomics.com/products
order@preomics.com
Tel: +49-89-2314-1630

Figure 1

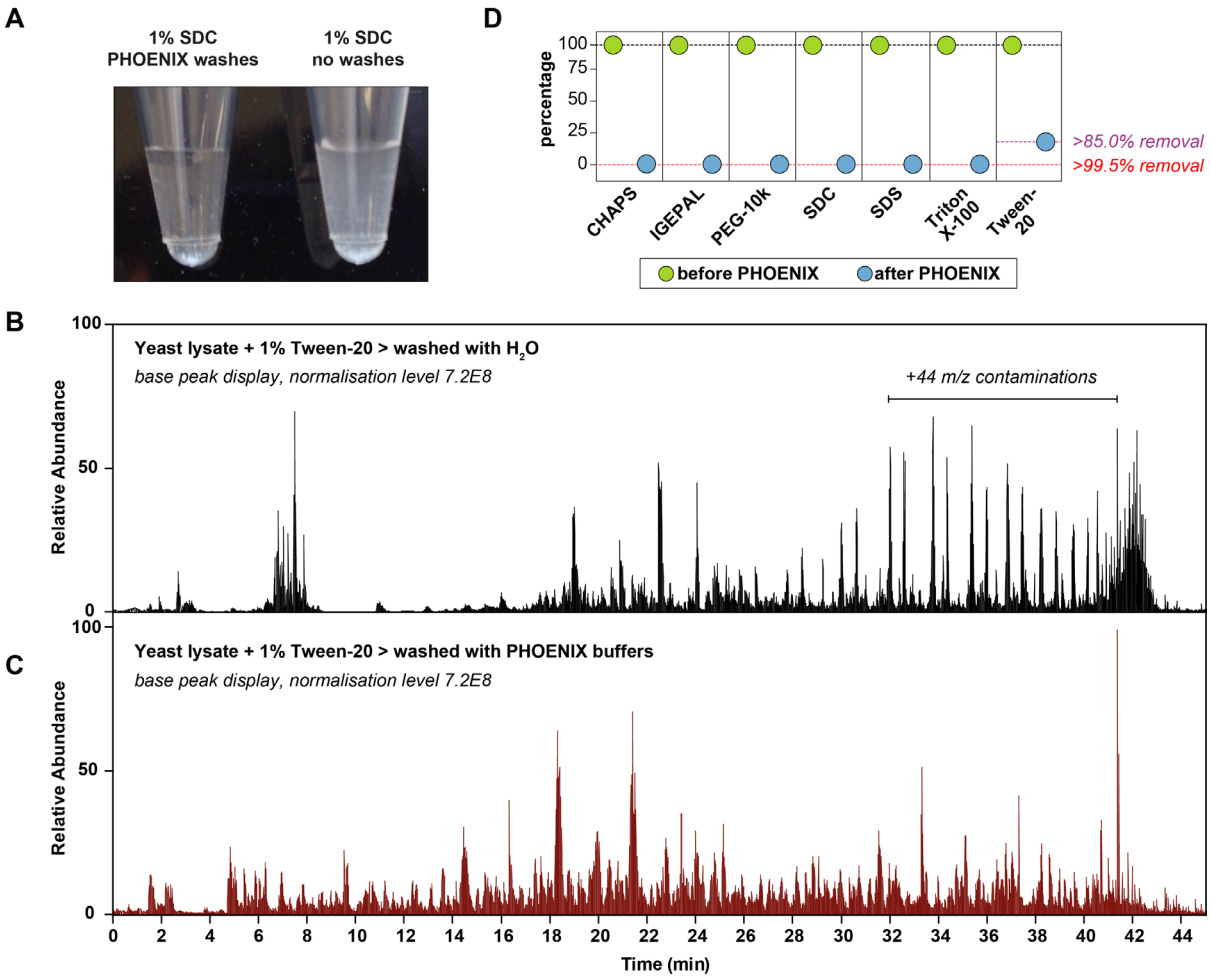

Figure 1

(A) Photograph of yeast lysates with 1% SDC spiked-in followed by Phoenix washes (left) or no washes (right). **(B+C)** Base peak display of selected raw files, normalized relative abundances. Yeast lysates with 1% Tween-20 spiked-in followed by (B) H_2O wash controls or (C) Phoenix washes. **(D)** Removal efficiency of Phoenix washes for several commonly used detergents.

Figure 2

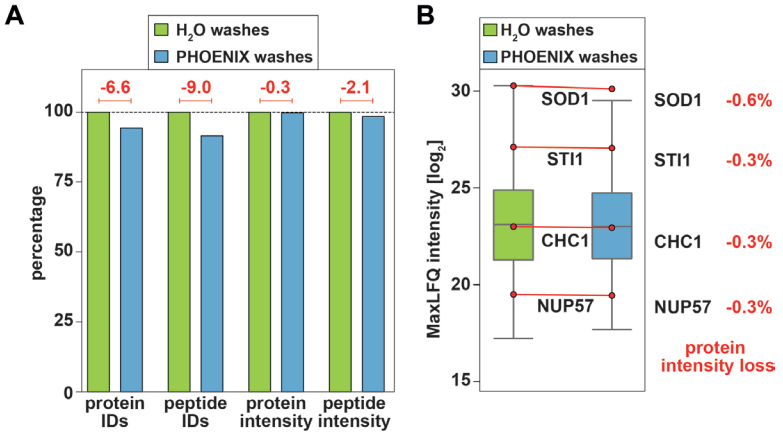

Figure 2

(A) Recovery of protein and peptide features after washing yeast lysates with Phoenix wash buffers or H_2O wash controls. Losses in protein and peptide identifications or protein and peptide intensities are indicated in red.

(B) Losses of protein intensities for four selected proteins spanning the global protein abundance range are indicated in red.