Phoenix Peptide Cleanup Kit

PreOmics Phoenix kit can clean up peptide mixtures removing detergents, polymers, salts, lipids to give reliable and robust LC-MS analyses.

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 using the iST Sample Preparation Kit (P.O.00001, PreOmics GmbH).

The tryptic peptide solutions were mixed with 1% of the selected detergents listed in table1.

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)

Table 1 | List of detergents tested with the Phoenix cartridge

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The peptide/detergent mix was then transferred to the Phoenix CARTRIDGE and washed according to the protocol of the Phoenix Peptide Clean up Kit (P.O.00023, PreOmics GmbH). LC-MS analysis was performed as described[1] on an Orbitrap XL mass spectrometer (ThermoFisher 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.
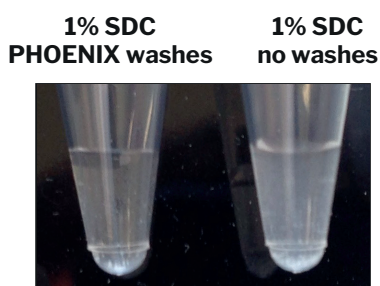
1% SDC
PHOENIX washes   **1% SDC**
no washes

Figure 1 | Visual inspection of the sample before and after using the Phoenix kit. Left tube shows sample color after using the phoenix kit, right tube shows sample color before Phoenix kit was used.

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 (Table 2 below). 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

Table 2 | Phoenix kit detergent removal efficiency

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Conclusion

Clean peptide samples, without detergent contamination, greatly increase HPLC column lifetime and prevent mass spectrometry downtime. Here, we demonstrate that the Phoenix Peptide Clean-up Kit removes detergents with good efficiency. The Phoenix kit is fully compatible with the iST sample preparation kit and downstream LC-MS/MS analyses, the kit can be automated using PreOmics PreON and other automation platforms.

References

(1) NA Kulak, G Pichler, I Paron, Nagaraj N and M Mann.
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(2) S Tyanova, T Temu and J Cox.
The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. Nat Protocols (2016) 11:2301-19. doi: 10.1038/nprot.2016.136.
(3) S Tyanova, T Temu, P Sinitcyn, A Carlson, MY Hein, T Geiger, M Mann and J Cox.
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
Phoenix Kit 96x	96 reactions	PreOmics GmbH	P.O.00023

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