Efficiency assay of detergent removal columns on protein and peptide samples for mass spectrometric analysis

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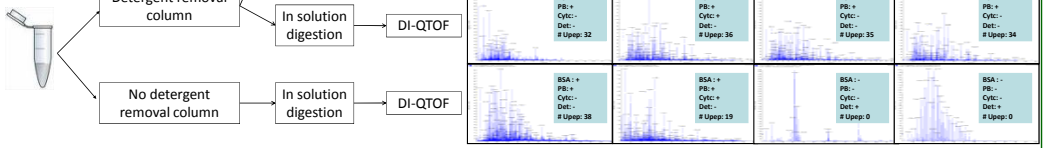
Introduction: Detergents are essential for protein solubility during protein extraction and sample preparation, especially when working with hydrophobic proteins (e.g. proteins tightly bound to cell membranes). However, the presence of high concentrations of detergents in protein samples can impair protease digestion of proteins and suppress peptide ionization when analyzed by mass spectrometry. New (GB-S10) detergent removal columns from G-Biosciences were tested for their efficiency to remove anionic, nonionic or zwitterionic detergents (e.g. SDS, TritonX100 or CHAPS) from protein and tryptic peptide samples with minimal sample loss for downstream analysis by mass spectrometry and other techniques.

Detergent Removal from Protein Samples

Protein mixture:
BSA (MW: 69 kDa, pI: 5.8)
PB (MW: 97 kDa, pI: 6.8)
CytC (MW: 12 kDa, pI: 9.6)

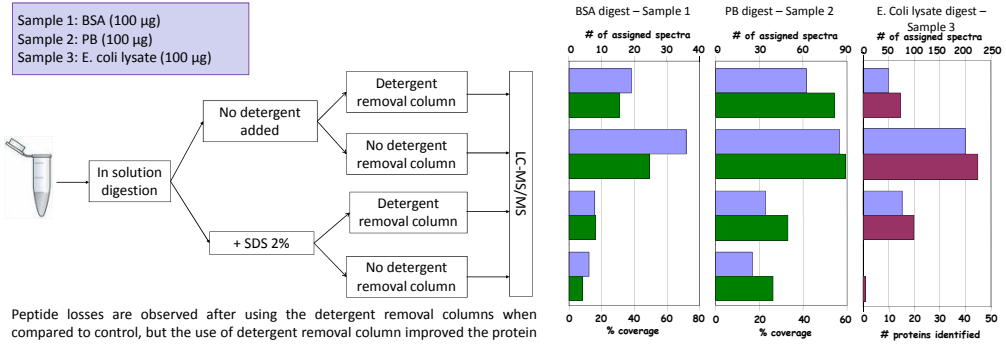


Control: 5 µg/µL protein mixture in water
+ SDS: Control in SDS 0.2%
+ CHAPS: Control in CHAPS 0.5%
+ Triton: Control in Triton X100 0.2%

Without using detergent removal column, the protein digestion was impaired by the presence of detergent, except for SDS because of the low concentration of use. We observed an increase of peptides matched after using the detergent removal column for the SDS sample, and almost no loss of peptides when compared with the control sample.

Detergent Removal of High Concentrations of SDS from Simple and Complex Tryptic Peptide Samples

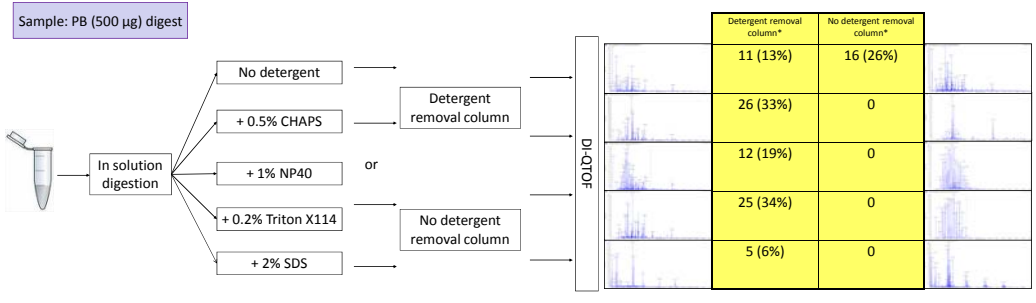
Sample 1: BSA (100 µg)
Sample 2: PB (100 µg)
Sample 3: E. coli lysate (100 µg)



Peptide losses are observed after using the detergent removal columns when compared to control, but the use of detergent removal column improved the protein recovery by increasing the number of peptides identified in samples when SDS is present.

Detergent Removal of Various Detergents from Tryptic Peptide Samples

Sample: PB (500 µg) digest



Detergent removal column*	No detergent removal column*
11 (13%)	16 (26%)
26 (33%)	0
12 (19%)	0
25 (34%)	0
5 (6%)	0

* Number of assigned spectra and % of sequence coverage

SDS (anionic), CHAPS (zwitterionic), NP40 (nonionic) and Triton X114 (nonionic) were sufficiently removed using the detergent removal column to increase peptide recovery and percentage of sequence coverage.

Summary:

Questions	0.2% SDS (anionic)	0.5% CHAPS (zwitterionic)	0.2% Triton X100 (nonionic)
Protein recovery	✓	✓	✓
Detergent removal from protein sample	✓	✓	✓
Protein electrophoresis	✓	✓	✓
Protein digestion	✓	✓	✓

Questions	2% SDS (anionic)	0.5% CHAPS (zwitterionic)	1% NP40 (nonionic)	0.2% Triton X114 (nonionic)
Peptide recovery	✓	✓	✓	✓
Detergent removal from peptide sample	✓	✓	✓	✓

Methods

OFFGEL electrophoresis and SDS-PAGE: 100 µg samples were separated on 4-7 pH strip, 12 fractions using the 3100 OFFGEL fractionator (Agilent). After applying 20 kV, fractions were collected and 1/3 was dried down and run via SDS-PAGE. The gel was stained with Sypro.

In solution digestion: Samples were reduced and alkylated before trypsin digestion.

DI-QTOF: Samples were resuspended in 5% ACN/ 0.1% FA, ziptipped using C18, and infused using nanospray tips into an ABI QSTAR XL (Applied Biosystems/MDS Sciex) hybrid QTOF MS/MS mass spectrometer. TOF mass and product ion spectra were acquired using information dependent data acquisition (IDA) in Analyst QS v1.1 with the following parameters: mass ranges for TOF MS and MS/MS were m/z 300-2000 and 70-2000, respectively. Every second, a TOF MS precursor ion spectrum was accumulated, followed by three product ion spectra, each for 3 s.

LC-MS/MS: Nano-LC was performed with an nano 2D LC (Eksigent) equipped with a Dionex C18 PepMap100 column (75 µm i.d.) flowing at 200 nL/min. Peptides (5 µL injections) were resolved on a gradient from 90.5% solvent A (0.1% FA in MilliQ water) and 9.5% solvent B (0.1% FA in ACN) to 25% B in 4 minutes, then increasing to 40% B over 75 minutes, and from 40-90.5% B over the final 5 minutes. Mass spectrometer parameters are identical to those described above.

Database searching: The peptide tandem mass spectra were searched against NCBInr using an in-house version of MASCOT v2.2 (Matrix Science Inc). The following parameters were selected: tryptic peptides with ≤1 missed cleavage site; precursor and MS/MS fragment ion mass tolerance of 0.8 and 0.8 Da, respectively; fixed carbamidomethylation of cysteine; and variable oxidation of methionine. Data was then compiled in Scaffold (Proteome Software). Positive identification was determined based on the following criteria: ≥2 peptide sequences, minimum peptide probability of 50% and minimum protein probability of 99%.