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Measuring Sodium Dodecyl Sulfate Carryover In Protein Hydrolysates Prepared via Protein Aggregation Capture

Abstract

The need for an automatable solution to LC-MS sample preparation has given rise to the Covaris Protein Aggregation Capture (PAC) workflow [1,2]. To improve protein extraction efficiency, especially from FFPE tissue samples, our process utilizes the detergent Sodium Dodecyl Sulfate (SDS). Concern for detergent carryover is warranted due to the relatively low tolerable limit of 0.01% for most applications that use LC-MS for detection, identification, and quantification. A highly sensitive method for the detection of SDS in aqueous solutions was utilized to examine the potential contamination of nucleic acid and peptide eluates. SDS concentrations in the LC-MS ready peptide hydrolysates were below 0.0001% which is 2 orders of magnitude below the allowable limit for LC-MS processing. These data suggest that the Covaris PAC workflow, which applies Adaptive Focused Acoustics® (AFA®) Technology at various steps in the protocol, produces exceptionally pure protein hydrolysates without significant carryover of SDS.

Introduction

The Covaris PAC workflow is a method for extracting and purifying nucleic acids and liquid chromatography mass spectrometry (LC-MS) ready peptide digests from cultured cells and human tissue. The PAC process was originally designed to function as a protein isolation and purification technique that could be performed in a single vessel. The workflow utilizes paramagnetic beads to indiscriminately bind proteins and to enable the removal of harsh chemicals which are often essential for lysis and solubilization. PAC is highly compatible with a wide range of sample inputs as well as detergents, chaotropes, salts, and solvents [3]. Proteolytic cleavage is performed on-bead, and allows for highly efficient and unbiased sample recovery.

The Covaris PAC workflow requires minimal liquid transfer; the workflow starting from cell culture and deparaffinized FFPE tissue can be performed in a single plate format and is fully automatable. The extraction process is facilitated through the use of lysis buffers which contain chaotropic agents or detergents like sodium dodecyl sulfate. While PAC is reputed to generate samples with a high degree of purity, carryover of SDS into the final peptide lysate remains a possible concern due to its suppressive effects on analyte ion signal and fouling of equipment during LC-MS [4].

A method was published describing the colorimetric quantitation of SDS in biological samples [5]. The reported approach relies on measuring the interaction of SDS with the extraction efficiency of methylene blue into chloroform. This application note describes the quantitation of SDS carryover in final tryptic digests of the Covaris PAC workflow to determine if they contain any significant degree of contaminating detergent.

Material & Methods

Materials

- NanoDrop® Spectrophotometer (Thermo Fisher Scientific Inc.®, ND-2000)
- Balance (Sartorius AG®, BCE124-1S)
- Vortex (Scientific Industries Inc.®, SI-0236)
- Centrifuge (Eppendorf®, 022620444)

Consumables

- 0.5 mL Centrifuge Tubes (Eppendorf, 022363719)

Materials

- Methylene BlueChloride (MilliporeSigma®, 284-12)
- Sodium Sulfate (MilliporeSigma, 71959)
- Nuclease Free Water (Invitrogen®, AM9932)
- Sulfuric Acid (MilliporeSigma, 258105)
- Chloroform (MilliporeSigma, 496189)
- Sodium Dodecyl Sulfate (MilliporeSigma, 71736)

Methods

Adherent cells (MCF7) and suspension cells (K562) were cultured and harvested prior to AFA-mediated cell lysis. Lysates were reduced, alkylated, and then purified via protein aggregation capture. RNA was eluted after purification, and proteins were hydrolyzed on-bead with AFA-enhanced trypsin digestion. Starting with cell culture, the Covaris PAC workflow is depicted in ***Figure 1***.

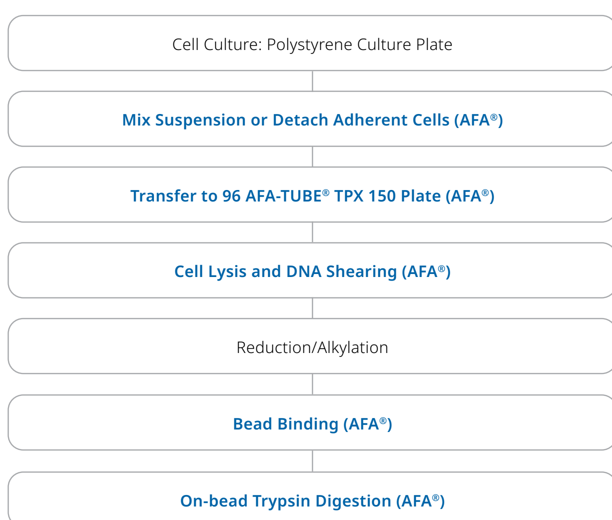

Figure 1. Covaris PAC workflow. AFA is utilized to detach adherent cells without trypsinization, lyse cells and shear DNA to reduce sample viscosity, bind protein and nucleic acid to magnetic beads, and finally to execute trypsin digestion.

Standards were prepared through serial dilution of a 1% SDS stock solution. Optimal SDS concentrations for a standard curve were determined to be 0.01%, 0.007%, 0.004%, 0.001%, 0.00005%. Thirty (30) µL of either SDS dilution or peptide tryptic digest was mixed with 30 µL of methylene blue reagent (250 mg methylene blue, 50 g Na2SO4, 10 mL H2SO4 per liter of water) in a 0.5 mL Eppendorf tube. A 120 µL aliquot of chloroform was added, and tubes were vortexed for 10 seconds at maximum speed. Samples were then centrifuged at 16,000 x g for 1 minute. The lower organic phase was transferred into a new 0.5 mL Eppendorf tube containing 20 mg anhydrous sodium sulfate. Tubes were vortexed for 10 seconds, and then centrifuged at 16,000 x g for 1 minute. NanoDrop measurements were taken at an absorbance of 651 nm using a 4 µL aliquot of the chloroform supernatant.

Results

Concentrations of contaminating SDS were calculated for each peptide sample by fitting absorbance values to a standard curve (***Figure 2***). The standard Covaris PAC workflow was compared to a modified version of the workflow with two additional wash steps using 24 replicates for each condition. No significant difference in detergent concentration was detected (***Table 1***). The standard and modified versions of the Covaris PAC workflow were further evaluated through the comparison of low and high cell counts. Both the low (104) and high (106) cell count sample eluates contained similarly low levels of contamination (***Table 1***). Finally, the workflows were tested via the addition of up to 9 mg of paraffin alongside cells prior to PAC to mimic more challenging sample types such as Formalin Fixed Paraffin Embedded (FFPE) tissue. Wax appeared to have only a very minor effect on carryover of SDS (***Table 1***).

Table 1. Changes in detergent concentration of PAC workflow eluates.

Condition	SDS (%)	St. Dev.
Standard Workflow	0.000019	0.000021
Modified Workflow	0.000015	0.000016
Low Cell Count	0.000023	0.000010
High Cell Count	0.000051	0.000013
3 mg Paraffin	0.000074	0.000015
6 mg Paraffin	0.000068	0.000014
9 mg Paraffin	0.000093	0.000011

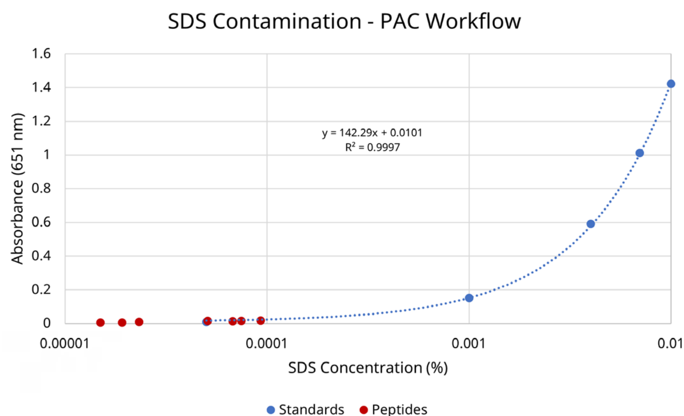

Figure 2. Summary of SDS contamination measurements. Calculated SDS concentrations of peptide eluates purified using the Covaris PAC workflow were plotted alongside standards.

Conclusion

No significant SDS contamination was detected in any eluate resulting from the Covaris PAC workflow. Most samples contained negligible amounts of detergent that were at or below the detection limit of this assay. Any sample with an appreciable amount of SDS was determined to contain below 0.0001% of the detergent which is 100 times below the acceptable limit for LC-MS. The Covaris PAC protocol has been extensively tested to confirm that peptide eluates are sufficiently pure and free of detergent contamination. Following this protocol will ensure that LC-MS analysis will not be impacted by SDS carryover.

References

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