ANCHOR MOLECULAR

Memorandum

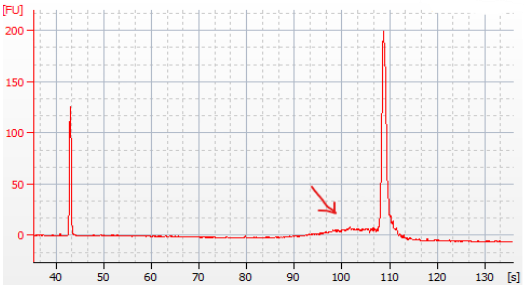
To: Customer of DNA-Depleted Plasma
From: Anchor Molecular R&D
Date: Oct 27, 2024
Re: High MW "DNA" Band from cfDNA Extraction of DNA-Depleted Plasma

Purpose

To demonstrate that the observed high MW band from silica bead-based cfDNA extraction of DNA-depleted plasma is plasma protein, not residue DNA. To provide the basis for proteinase treatment before performing accurate DNA quantification.

Background

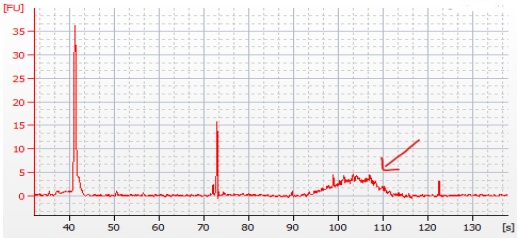
After silica bead-based cfDNA extraction of DNA-depleted plasma, it is sometimes observed that the eluent shows high absorbance in fluorescence-based assays (such as Qubit or DeNovix DNA assays). On the Bioanalyzer, there is sometimes a broad high molecular weight band or bump with a variable size between 1000 bp to 20000 bp (between 100 to 120 seconds in retention time), near or at the high molecular marker.



The fluorescence signal sometimes significantly contributes to the DNA mass quantified by fluorescence-based DNA assays, leading to questions about whether the DNA depletion is extensive enough. We have performed a series of experiments to show that **the high molecular weight band is protein, not DNA**.

Experimental data

1. The high molecular "DNA" band is resistant to DNases after treatment with either DNase I or MNase: the band remains after treatment.



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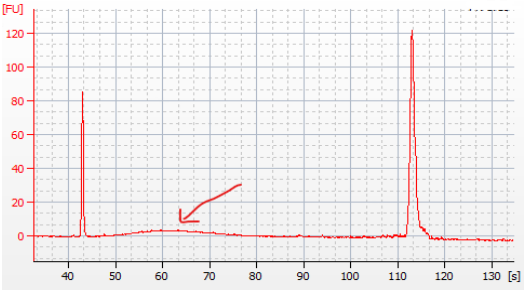
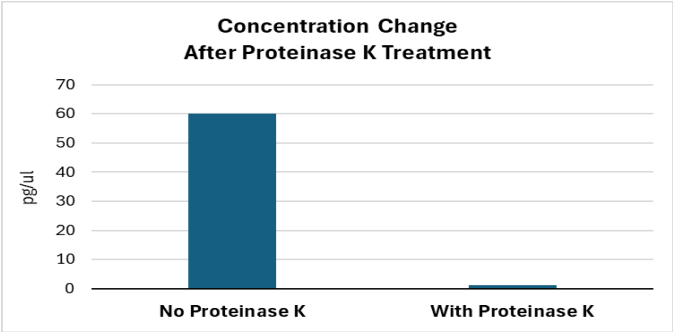
2. The high molecular “DNA” band was removed by proteinase K (and the peak size shifted to low molecular weight which corresponds to degraded peptides).



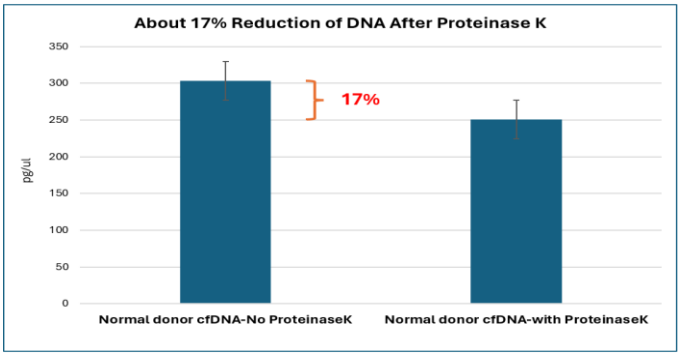
The band between 100-110s was removed and a band was seen at faster retention time (smaller size) indicating the broken down of the high molecular weight molecules.

3. DeNovix data shows the florescence signal was abolished by added proteinase K.



The DNase-free proteinase K used does not affect true cfDNA concentrations from chromatographically purified cfDNA (data available upon request).

4. Protein contamination is commonly observed in bead-based cfDNA extraction of patient plasma cfDNA: proteinase K caused reduction of cfDNA concentration extracted from normal plasma donor:



Conclusion

The observed high MW “DNA” band from bead-based cfDNA extraction of DNA-Depleted Plasma is from plasma protein, not from residue DNA. We will perform pre-treatment of proteinase K to the extracted cfDNA before it is used for fluorescence-based DNA analysis.