Phoseon™ TECHNOLOGY

An EXCELITAS TECHNOLOGIES Company

Real-time Analysis of Nucleic Acid Purity

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Conclusion

The KeyView Prep™ system allows for real-time 260/280 analysis that is both efficient and accurate.

Introduction

Since its conception in the 1940s, the 260/280 assessment of nucleic acid purity has routinely relied on spectrophotometric analysis and well-designed blanks. When implemented properly these analyses provide adequate quantity and quality assessments that inform downstream applications. Often, however, inherent biases in these protocols have misrepresented sample purity and negatively impacted downstream efforts.

Two key biases seen in current 260/280 assessments are control mismatches and equipment differences. If the control solution does not closely match the sample in both ionic strength and pH the resulting over- or under-representation of purity undermines any subsequent analysis of that sample. Minor difference in wavelength (particularly at 280nm) can similarly invalidate further testing. Significant ratio differences have been seen even when comparing spectrophotometers that all lay within the 1nm wavelength specification. An issue germane to both intra- and inter-laboratory comparisons of the same sample. Here we report a novel, real-time 260/280 assessment of nucleic acid purity that is aimed at minimizing these inherent biases.

Methods

- ### Phase #1 – Pure Samples

  20mL of salmon sperm DNA (0.126mg/mL, Sigma Aldrich, SKU# D1626) and 20mL of Bovine Serum Albumin (BSA, 1.25mg/mL, Sigma Aldrich, SKU# 05470) were individually injected into the KeyView Prep system (Phoseon Technology) at a flow rate of 5mL/min. After each injection the system was flushed and needle port cleaned and a new run was initiated. Response (AU) was recorded at all six channels in the system (220nm, 255nm, 265nm, 280nm, 285nm and 297nm) simultaneously. The response at each wavelength was then divided by the 280nm response (AU).

- ## Phase #2 – Protein/Nucleic Acid Mixtures

Dilution #	% BSA	% DNA
1	100	0
2	90	10
3	70	30
4	30	70
5	10	90
6	0	100

Stock concentrations of BSA (0.417mg/mL) and DNA (0.0421mg/mL) were prepared and subsequently mixed in varying ratios. Each mixture contained a total of 20mL. The table on the left shows what ratios of BSA to DNA were contained in each solution.

Again, response (AU) was recorded at all six channels in the system (220nm, 255nm, 265nm, 280nm, 285nm and 297nm) simultaneously. The response at each wavelength was then divided by the 280nm response (AU).

Results: Phase 1 of testing involved pure samples of both BSA and DNA (see Figure 1 below). As expected BSA showed a 255/280 ratio of 0.63; well within margin of 0.57 expected for pure proteins. Results for DNA were also well within the expected range at a ratio of 1.81 (the expected ratio for pure DNA is 1.8-2.0).

A

Channel	Peak height (AU) / 280nm (AU)	
	BSA	DNS
220	14.89	1.08
255	0.63	1.81
265	0.77	1.71
280	1.00	1.00
285	0.76	0.72
297	0.25	0.22

B

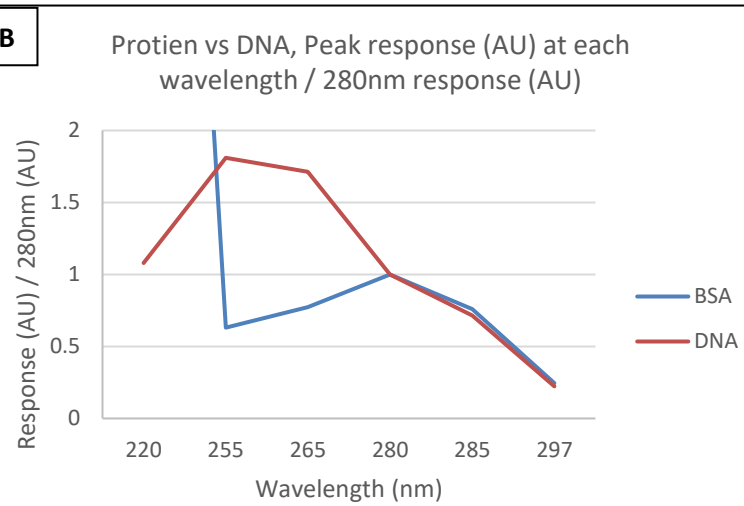

Figure 1: Pure BSA and DNA at each wavelength

20mL of BSA (1.25mg/mL) and DNA (0.126mg/mL) were independently injected at a flow rate of 5mL/min. All six channels of KeyView Prep were recorded simultaneously and the response (in AU) at each channel was then divided by the response (AU) at 280nm. Figure A is a tabular depiction of the Response (AU)/280nm (AU) data and figure B is a graphical depiction. As expected, the pure DNA shows a 255/280 ratio of 1.81 and the pure BSA shows a ratio of 0.63.

NOTE: The 220nm channel was maxed out by the BSA dilution and showed a 220/280 ratio of 14.89. As such it was included in the graph (Figure 1B) however the scale was adjusted so as to better view the Response/280 ratios for the rest of the channels.

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Phase 2 of testing involved increasing ratios of DNA to BSA, starting with 0% DNA (100% BSA) and ending with 100% DNA (0% BSA). Stock solutions of BSA (0.417mg/mL) and DNA (0.0421mg/mL) were mixed in various ratios and analyzed in succession in the same run. As shown in Figure 2 below, the 255/280 ratio increased incrementally from 0.67 to 1.63 as the percentage of DNA increased and the percentage of BSA decreased. It is important to note that the ratios for pure protein (dilution #1) and pure DNA (dilution #6) were both approximately 10% different from the original values seen in the first phase of testing (see Figure 1). This is not concerning, however, as the concentrations of both solutions were 3X lower in the second phase than the first phase.

Discussion

Development of this real-time 260/280 protocol was completed in two phases. In the first phase we examined purportedly pure nucleic acid and protein samples. Both results were within the margin expected for "pure" BSA and DNA samples, thus signifying the efficacy of this real-time analysis. Once the validity of this method was confirmed we next turned to mixed protein/nucleic acid solutions.

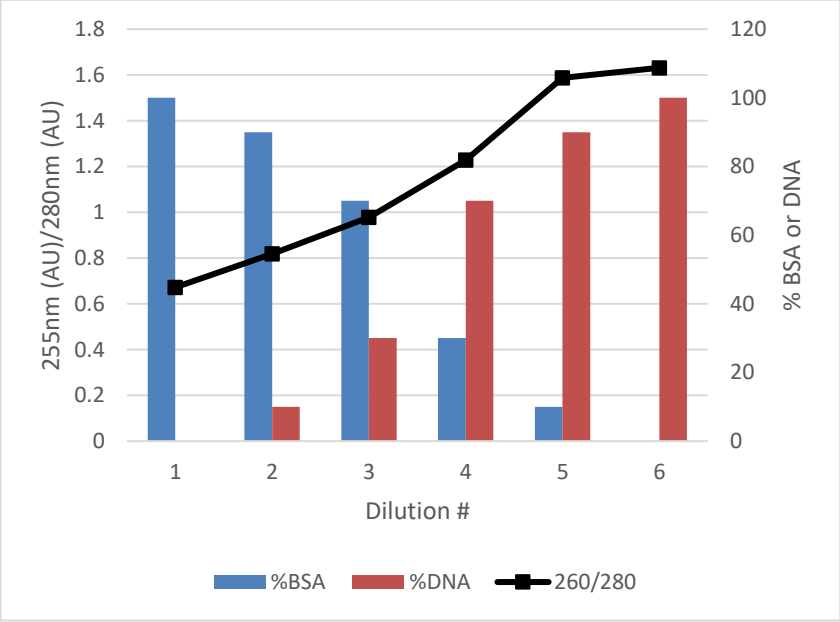

Figure 2: 255/280 Ratio at Varying BSA and DNA mixtures
Stock concentrations of BSA (0.417mg/mL) and DNA (0.0421mg/mL) were prepared. From the stock solutions six different mixtures were prepared, each containing a total of 20mL. Each dilution was then injected into the KeyView Prep system (Phoseon Technology) at a flow rate of 5mL/min, starting with dilution #1 and ending with dilution #6. All six channels were simultaneously recorded during the run, however only the 255 and 280 channels are represented here.
The response (AU) of the 255nm channel was divided by the 280nm response (AU). As expected, pure BSA showed a ratio of 0.67, with the ratio increasing as the percentage of DNA increased and ending with pure DNA at a ratio of 1.63.

In the next phase of testing we assessed the sensitivity of this new method by examining if it could be used to determine when there is a mixture of DNA and protein rather than a pure sample. Here we see incremental increases in the 260/280 ratio as the amount of DNA increased and BSA decreased. Notably, we see a measureable change in the 260/280 ratio when going from dilution 6 to 5 (0:100 and 10:90, %BSA:%DNA, respectively). It is common knowledge that the 260:280 ratio does not appreciably change until there is at least 75% protein contamination. We were able to detect a difference, however, after only 10% protein

contamination (see Figure 2). This could potentially signify greater sensitivity of real-time analyses as compared to currently used static spectrophotometric analysis. Further testing is planned to detail this trend.

Ultimately, we have shown that it is not only feasible to complete 260/280 analysis in real-time with KeyView Prep, but also expeditious and practical. By measuring under flow conditions we remove the need for well-designed controls and other time-consuming steps currently utilized. We also minimize instrumental wavelength drift as the same instrument would be used for nucleic acid separation and purity testing.

Literature Cited

1. Lucena-Aguilar, G., Sánchez-López, A. M., Barberán-Aceituno, C., Carrillo-Ávila, J. A., López-Guerrero, J. A., & Aguilar-Quesada, R. (2016). DNA Source Selection for Downstream Applications Based on DNA Quality Indicators Analysis. Biopreservation and biobanking, 14(4), 264–270. https://doi.org/10.1089/bio.2015.0064

2. Wilfinger, W.W, Mackey, K, Chomczynski, P. (1997). DNA Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity. BioTechniques, 22(3), 474-481.

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