Protein Measurement Accuracy and Reproducibility

Introduction

Although the use of Thermo Scientific NanoDrop Spectrophotometers for nucleic acid quantification is well established in the life science community, it is less well known that these instruments are also capable of quantifying purified proteins at 280 nm with the same high degree of accuracy and reproducibility. The auto-ranging pathlength capability of the NanoDrop™ 2000/2000c allows for the quantification of a very broad range of sample concentrations without the need for dilution. To demonstrate this, accuracy and reproducibility of the NanoDrop 2000c for protein quantification were evaluated using a dilution series of bovine serum albumin (BSA).

Method

A serial dilution of BSA in PBS was prepared and quantified using both the NanoDrop 2000c and a reference spectrophotometer. Each concentration was measured ten times on the NanoDrop 2000c and three times on the reference spectrophotometer. While the NanoDrop 2000c was able to measure all dilutions using only 2 µL per measurement on the pedestal, the reference spectrophotometer required larger volumes and specialized cuvettes to cover the same range.

Calculations of protein concentration from absorbance values were automatically performed by the NanoDrop 2000c software. Absorbance data was exported from the reference spectrophotometer and protein concentrations manually calculated according to Beer's Law using an E1% of 6.67.

Results

The NanoDrop 2000c displayed excellent accuracy at all concentrations tested (fig. 1). The upper detection limit for protein samples will vary depending on the type of protein being measured. For example, the dynamic range of the instrument when measuring BSA using the pedestal option is 0.1 – 400 mg/mL. The upper detection limit of a spectrophotometer using a standard 10 mm cuvette is approximately 200 fold less than that of a NanoDrop 2000c when using the pedestal option. The extended dynamic range of the NanoDrop 2000c enables measurement of protein samples regardless of concentration, whereas cuvette-based spectrophotometers require some prior knowledge of protein concentration to ensure the appropriate sized cuvette is used.

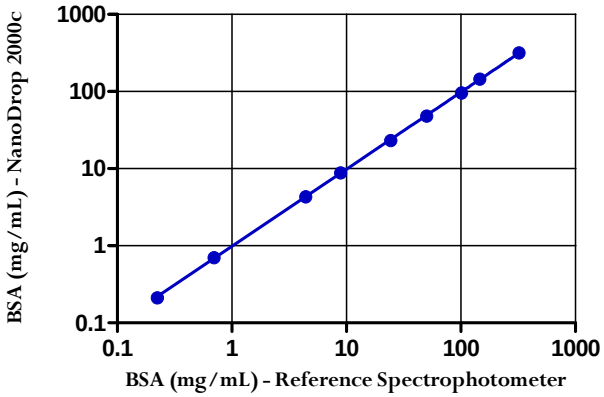

Figure 1: Accuracy of NanoDrop 2000c (n=10) vs. reference spectrophotometer (n=3).

Published reproducibility for the NanoDrop 2000c is a standard deviation of 0.1 mg/mL for BSA concentrations less than 10 mg/mL. Above 10 mg/mL BSA, reproducibility is typically less than a CV of 2 %. In this study, the NanoDrop 2000c far exceeded these specifications (table 1).

Concentration (mg/mL)	SD	%CV
0.21	0.02	N/A
0.70	0.01	N/A
4.33	0.01	N/A
8.82	0.02	0.21
23.10	0.07	0.31
48.04	0.12	0.25
95.89	0.37	0.38
145.62	0.68	0.47
316.45	1.18	0.37

Table 1: Reproducibility of NanoDrop 2000c pedestal measurements. For each concentration, n=10

The NanoDrop 2000c displayed excellent accuracy and reproducibility throughout the concentration range tested. In addition to this, the use of a NanoDrop spectrophotometer for direct measurement of protein concentration at 280 nm presents significant savings in time and reduces the opportunity for human error compared to cuvette-based spectrophotometers.

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