Microvolume Protein Performance Data

Technical Note 107

Introduction

The 1 mm pathlength used by some microvolume spectrophotometers work well enough for nucleic acid measurements. However, this pathlength presents challenges when measuring purified proteins. The 0.5 mm path utilized by the next generation DeNovix® DS-11 microvolume spectrophotometer has addressed this issue making it the ideal instrument for the quantitation of a broad concentrated range of protein samples.

Spectrophotometers are evaluated based on their precision and linearity. Precision describes the reproducibility of measurements on a spectrophotometer. The DS-11 SmartPath® Technology enables highly reproducible measurements with an absorbance precision of 0.0013 AU (0.5 mm path) at absorbances below 6 mg/mL BSA and 1% or less variance at absorbances greater than 6 mg/mL BSA.

Linearity is an assessment of a spectrophotometer's working range. The DS-11 is capable of reading absorbance values from 0.015 A to 750 A (1 cm equivalent). This translates to a lower limit of 0.04 mg/mL BSA or 0.02 mg/mL IgG and an upper limit of 1125 mg/mL BSA or 548 mg/mL IgG. This broad range virtually ensures that diluting protein samples will be unnecessary, thus eliminating time and reducing associated errors. The purpose of this technical bulletin is to present data that demonstrates the DS-11 meets the above stated specifications.

Materials

A series of dilutions was prepared to cover a large portion of the DS-11's linear range using a 300 mg/mL BSA stock (Sigma-Aldrich cat # A7284) diluted in 1x sterile filtered phosphate buffered saline (PBS) (Pierce cat #28372). Reference concentrations were determined using the 280 nm absorbance values obtained on an Agilent 8453 reference spectrophotometer (Agilent, Santa Clara CA) in a 1 mm quartz cuvette (Starna, cat #1-Q-1). The absorbance value along with an E1% value of 6.67 were used in the Beer-Lambert equation to calculate sample concentrations.

Methods

The upper absorbance range of the Agilent spectrophotometer is ~2.0 A, which is equivalent to 30 mg/mL BSA. The reference values for test solutions above this limit were determined by gravimetrically diluting the samples to fall within the Agilent's range.

The Protein A280 app was launched and a microvolume mode Blank measurement was made using 1.5 µL of PBS. Five measurements were then made for each sample concentration. Fresh 1.5 µL aliquots were used for each replicate measurement. The sample solution was removed between each measurement by wiping the upper and lower sample surfaces with a clean dry laboratory wipe.

Precision Results

A standard deviation of +/- 0.04 mg/mL for BSA samples < 3 mg/mL meets the specified absorbance precision of 0.0013 AU (0.5 mm path). In addition, the reported % CV of 1% or less meets specified absorbance precision for samples BSA > 3 mg/mL.

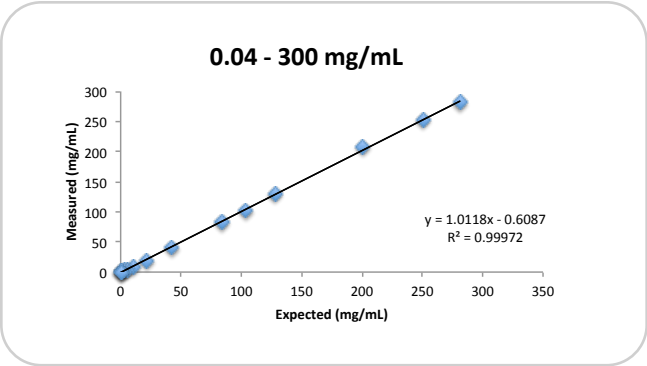
Table 1: DS-11 Precision

Expected mg/mL	Average mg/mL	StDev	%CV
0.045	0.044	0.0048	11.1
0.109	0.104	0.0052	5.0
0.500	0.495	0.015	3.1
1.08	1.02	0.036	3.5
3.05	2.98	0.025	0.83
21.5	20.0	0.063	0.32
42.6	39.8	0.116	0.29
128.8	129.0	0.488	0.38
201.2	207.5	1.053	0.51
250.4	252.8	0.802	0.32
281.0	281.8	0.698	0.24

Linearity Results

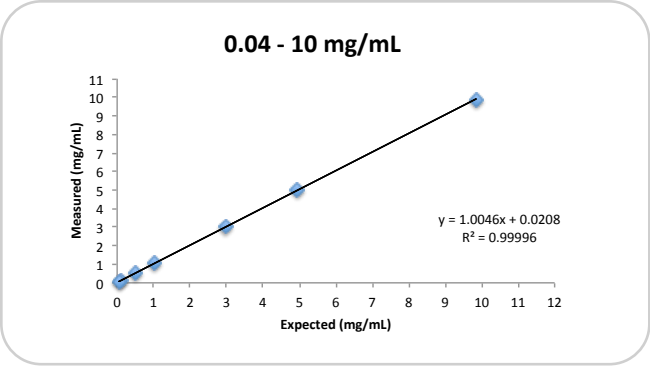
The linear response of the DS-11 spectrophotometer for protein samples is presented below. Target absorbances were determined using an Agilent 8453 reference spectrophotometer. The first graph (Fig. 1) shows the linearity results for the full concentration range tested. The dilutions series chosen for this study highlight the DS-11's ability to measure highly concentrated samples.

Figure 1: DS-11 Linearity (Full Range)



The second graph (Fig. 2) shows the linearity results for just the lower concentration range to better visualize the outstanding linearity in this range.

Figure 2: DS-11 Linearity (Low Range)



Carryover

Protein samples are known to be more sticky than typical nucleic acid solutions. Some labs have chosen to restrict the use of other microvolume spectrophotometers to either nucleic acids or proteins out of concern of carryover. A study was performed to demonstrate that even very high concentration protein samples can be effectively removed from the DS-11 sample surfaces, eliminating the concern regarding carryover. For this study the sample surface was vigorously wiped after each of five measurements using an ultra high concentration BSA sample. Five replicates of PBS were then taken on the DS-11 with the expected results to be within the +/- 0.04 mg/ml lower detection limit of the instrument. The results are seen in Table 2 below.

Table 2: Carryover Study

Sample	Average mg/mL
BSA	313.89
PBS	-0.004

Summary

The data presented in this technical bulletin demonstrate that the DeNovix® DS-11 microvolume spectrophotometer meets the published precision and linear range specifications for protein samples. Additionally the SmartPath® Technology automatically uses multiple pathlengths to enable absorbance measurements across a broad linear range using only 1.5 µL aliquots per measurement. As shown in Figures 1 and 2, samples with concentrations over 4 orders of magnitude may be routinely analyzed without making dilutions or using special caps or cuvettes.

The ease of use coupled with the broad absorbance range capabilities of the DS-11 makes this the ideal microvolume spectrophotometer choice for routine laboratory quantification of purified protein samples.

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