

Microvolume Nucleic Acid Performance Data

Technical Note 105

Introduction

The precision specification associated with a spectrophotometer is an assessment of the reproducibility of measurements. The DeNovix® DS-11 microvolume SmartPath® Technology enables highly reproducible nucleic acid measurements with an absorbance precision of 0.002 AU (0.5 mm path) at concentrations below 200 ng/μL and 1% or less variance at concentrations greater than 200 ng/μL dsDNA.

Linearity specifications refer to the working range of a spectrophotometer. The DS-11 has a linear range from 0.015 A to 750 A (1 cm equivalent). This translates to 0.75 - 37,500 ng/μL dsDNA. The DS-11 offers outstanding accuracy within the broadest linear range of any spectrophotometer on the market today.

The purpose of this technical bulletin is to present data demonstrating that the DS-11 meets the above stated specifications. To highlight the precision and accuracy of the instrument over the entire linear range of the instrument, a dilution series of purified double stranded DNA was prepared and five replicates of each concentration was measured using the DS-11 spectrophotometer's dsDNA app.

Materials

A 30 mg/ml solution of dsDNA was gravimetrically prepared using biotechnology grade fish sperm DNA sodium salt (Amresco cat #1B1509-256) and HPLC grade water (Ricca cat #9153-1). A series of 11 dilutions ranging from 5 ng/μL to 24000 ng/μL was then prepared using the HPLC grade water.

Reference concentrations for the dilutions were determined using an Agilent 8453 (Agilent, Santa Clara CA) in a 1 mm quartz cuvette (Starna, cat #1-Q-1). The reference value for DNA solutions with absorbance values outside of the Agilent's upper range of 2.0 A (equivalent to 1000 ng/μL dsDNA) were determined by gravimetrically diluting these samples to fall within the linear range of the reference spectrophotometer.

Methods

The dsDNA app was launched and a microvolume mode Blank measurement was made using 1 μL of HPLC grade water. Five measurements were then made for each dsDNA sample concentration. Fresh 1 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. Concentrations are calculated by applying Beer's Law using 10 mm equivalent 260 nm absorbance values.

Precision Results

The standard deviation of +/- 2 ng/μL or less for dsDNA samples < 200 ng/μL presented in the table below meets the DS-11 specified absorbance precision of 0.002 AU (0.5 mm path). In addition, the reported % CVs of 1% or less meets the specified absorbance precision for samples > 200 ng/μL dsDNA.

Table 1: DS-11 Precision for dsDNA

Expected ng/μL	Average ng/μL	0.5 mm SD	%CV
5	4.7	0.0005	NA
10.62	10.93	0.0006	NA
52.33	53.05	0.0004	NA
103.11	104.66	0.0006	0.6
497.78	502.47	0.0007	0.13
1009.95	1002.33	0.0015	0.15
4435.37	4394.28	0.0162	0.37
9005	8824.63	0.0333	0.38
11976	11862.35	0.0868	0.73
14730	14566.33	0.1328	0.91
23263	22192.54	0.0946	0.43

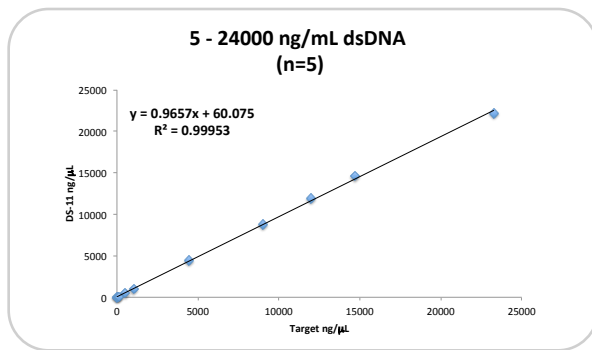


Linearity Results

Graphical representations of the broad linear range response of the DS-11 spectrophotometer are presented below. Target absorbances were determined using an Agilent 8453 reference spectrophotometer as previously described.

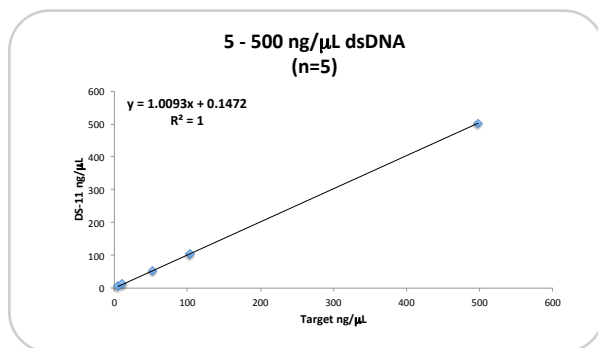
The first graph (Fig. 1) shows the linearity results for the full concentration range tested. The dsDNA concentrations chosen for this study highlight the DS-11's ability to measure nucleic samples with 10 mm equivalent 260 nm absorbance values up to 500 AU.

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)



Discussion

The data presented in Table 1 and graphically represented in Figures 1 and 2 demonstrate that the DS-11 microvolume spectrophotometer meets the published precision and linear range specifications on nucleic acid samples commonly used in molecular biology laboratories.

The SmartPath® Technology automatically uses multiple pathlengths to enable absorbance measurements across a broad linear range while conserving sample. Each microvolume measurement requires only a 1 μL aliquot of sample.

As shown in Figures 3a and 3b, the DS-11 microvolume mode makes it easy to measure samples with concentrations that span 4 orders of magnitude without making dilutions or using special caps or cuvettes.

Figures 3a and 3b: Microvolume Mode



The DS-11+ model, with its built-in cuvette capability, extends the lower detection range for nucleic samples down to 0.04 ng/μL.

Summary

The ease-of-use associated with the DeNovix DS-11 coupled with its outstanding precision and linearity over a broad absorbance range makes this the ideal microvolume spectrophotometer for nucleic acid quantification.

In addition, this versatile stand-alone instrument includes intuitive Android™ based software with EasyApps® for protein quantification and full spectrum UV-Vis analysis.