Microvolume Mode Carryover Studies

Technical Note 113

Introduction

Small volume spectrophotometric absorbance measurements have become the preferred method for nucleic acid and purified protein concentration determinations since the first microvolume instrument was introduced a decade ago. The simplicity of pipetting a 1 or 2 microliter sample onto a surface followed by a quick removal using a lab wipe eliminated the hassle of cleaning cuvettes between sample measurements. To take full advantage of this microvolume ease-of-use paradigm, it is important that the sample measurement surface design facilitates easy clean-up between samples and does not promote carry-over.

This technical note will present data demonstrating that the sapphire and quartz surfaces of the of the DeNovix® DS-11 spectrophotometer microvolume surfaces meet the requirements described above.

Method and Materials

The carryover of the DS-11 was assessed using both dsDNA (Affymetrix, cat # 14405) and bovine serum albumin (BSA) (Sigma Aldrich, cat # A7284). The first study assessed the carryover of a solution of ~ 5000 ng/µL dsDNA. The measurement sequence was as follows:

- 2 replicates of dH_20
- 3 replicates of dsDNA
- 2 replicates of dH_20

Fresh 1.0 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.

The second study assessed the carryover of a solution of ~20 mg/mL BSA The measurement sequence was as described above substituting the BSA for the nucleic acid sample and PBS for dH_20.

Results

Table 1: dsDNA and BSA Carryover

Sample	ng/µL	Sample	mg/ml
dH_20	0.00	PBS	-0.06
dH_20	-0.90	PBS	-0.05
dsDNA	5157.30	BSA	21.15
dsDNA	5078.20	BSA	20.94
dsDNA	5094.65	BSA	21.07
dH_20	0.65	PBS	-0.06
dH_20	0.90	PBS	-0.1

As seen in Table 1, the blank solution measured both before and after the nucleic acid and protein samples were below the DS-11 lower detection limit.

Ultra high concentration protein samples may require more rigorous wiping between samples. A study using a high concentration BSA sample showed a lack of carryover when surfaces were vigorously wiped using a dry lab wipe between BSA measurements. Subsequent PBS measurements met the expected results of being within the +/- 0.1 mg/ml lower detection limit of the instrument.

Table 2: Ultra High Concentration Carryover

Sample (n=5)	Average mg/mL
BSA	313.89
PBS	-0.004

Summary

The studies demonstrated a lack of significant carryover for either high concentrations of nucleic acid or protein samples. The DS-11 microvolume measurement surface facilitates easy clean-up to ensure minimal -to-no carryover of high concentration samples.

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