

# Sub-microliter DNA Analysis Using the nanoCell Accessory with an Evolution 300 Spectrophotometer

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## Introduction

In the life science laboratory, it is always desirable to get the maximum amount of information from the smallest volume of sample. DNA microarrays, real-time PCR, laser micro-dissected samples, and DNA sequencing for forensic analysis all rely on a very small volume of DNA solution, usually less than a milliliter, for analysis. Often these samples are very concentrated, requiring the ability to quantify DNA concentrations above 4,500 ng/ $\mu$ L. In this application note, we demonstrate the ability of the Evolution™ 300 UV-Visible spectrophotometer using the nanoCell accessory to analyze sub-microliter DNA samples in solution. We present data to illustrate the sensitivity of low-volume measurements, wide linear analysis range of the nanoCell accessory with the Evolution 300, reproducibility of multiple measurements, and the excellent carry-over performance of the nanoCell accessory from sample to sample.

## Sub-Microliter Measurements

The most convenient way to decrease the volume of solution required for a UV-Visible measurement is to reduce the pathlength. By reducing the pathlength from the traditional 1.0 centimeter to less than a millimeter, the volume of sample required for analysis can be reduced from milliliters to less than a microliter.

However, a shorter pathlength reduces the measured absorbance of the solution, thus a spectrophotometer with a wide dynamic range is required to make measurements over a large concentration range. The Evolution 300 spectrophotometer has a wide photometric range permitting accurate, linear results from 0.0005 to over 4.0 AU. This wide photometric range allows the nanoCell accessory to make accurate measurements of DNA concentrations from 5.0 ng/ $\mu$ L to 4,500 ng/ $\mu$ L using as little as 0.700 microliters of sample. The typical analysis range of nanoCell measurements made using the Evolution 300 for different genetic samples is given in Table 1.

	Calculation Factor	Small Volume Measurement Range (ng/ $\mu$ L)
dsDNA	50	5.0 – 4,500
ssDNA <sup>a</sup>	37	3.7 – 3,330
RNA <sup>a</sup>	40	4.0 – 3,600
Oligos <sup>a</sup>	30	3.0 – 2,700

<sup>a</sup> Results based on dsDNA

Table 1: Typical measurement range of the nanoCell accessory using a Evolution 300 spectrophotometer



The nanoCell accessory for the Evolution 300 spectrophotometer. The nanoCell is pictured with the 0.2 and 1.0 mm pathlength adapters.

## Flexible Sampling with the nanoCell

In addition to DNA concentration measurements, the Evolution 300 is a fully functional spectrophotometer designed for the life science laboratory. The local control software of the Evolution 300 allows wavelength scanning, fixed wavelength measurements, standard quantification assays, and kinetics measurements.

For convenience in the life science laboratory, the Evolution 300 offers over 20 pre-programmed assays for the analysis of DNA, RNA, and protein samples. These analyses are available in both Local Control and VISION<sup>pro</sup>™ software. These assays can be performed with a single drop of solution using the nanoCell accessory, or in a traditional cuvette. Having the ability to analyze sub-microliters or several milliliters of a sample provides the best sampling flexibility for your laboratory. The data acquisition and calculations for the following biological assays are automated, decreasing analysis time:

- DNA/Protein Concentration (260 & 280 nm with 320 nm correction)
- DNA/Protein Concentration with Scanning (260 & 280 nm with 320 nm correction)
- DNA Concentration (260 & 230 nm with 320 nm correction)
- DNA Concentration with Scanning (260 & 230 nm with 320 nm correction)
- dsDNA, ssDNA, RNA, and Oligos (260 nm with user-editable factor)
- Bradford Protein Concentration Assay – Standard and Micro
- Lowry Protein Concentration Assay – Standard and Micro

## Key Words

- Evolution 300
- nanoCell
- 260/280 Ratio
- DNA
- Small Volume Sampling
- Spectrophotometer
- Sub-microliter
- UV-Visible

## Experimental

A stock solution was prepared by dissolving calf thymus DNA in TRIS-EDTA (TE) buffer (10 mM TRIS, pH 7.4, 1.0 mM EDTA) to a final concentration of approximately 5 mg/mL. This solution was diluted serially to provide solutions of nominal DNA concentration from approximately 5000 to 5 ng/μL.

The equations used to estimate the DNA concentration and the DNA purity are shown below. The DNA concentration factor of 50 is based on the pathlength of a standard 1 cm cell and was adjusted for the pathlength used in the nanoCell measurements presented here.

$$\text{DNA Concentration} = \text{Abs}_{260} - \text{Abs}_{320} \times 50 \times \text{Pathlength Correction Factor}$$

$$\text{DNA purity} = \frac{\text{Abs}_{260} - \text{Abs}_{320}}{\text{Abs}_{280} - \text{Abs}_{320}}$$

The instrument was zeroed using 1.0 μL of TE buffer solution in the nanoCell accessory. Measurements were taken on a sample, the nanoCell sample area was cleaned with a Kimwipe®, and the next sample loaded. The sample area was cleaned only with a Kimwipe between samples, no washing of the sample area was necessary to remove the previous sample.

The measurements were made using an Evolution 300 spectrophotometer controlled with the VISION<sup>pro</sup> software package. Data was acquired in the fixed wavelength analysis mode. The absorbance at 260 nm, 280 nm, and a background absorbance at 320 nm was measured. The background at 320 nm was subtracted to determine the corrected 260 and 280 nm absorbance values. From these corrected values the DNA concentration and 260/280 ratio was determined.

All analyses were performed using a 1-second integration time and a spectral bandwidth of 2 nm.

## Linearity

The results from the DNA analysis using the nanoCell accessory on an Evolution 300 are shown in Figure 1 below. The linearity of the analysis is excellent, with a linear correlation coefficient of 0.99996. Each data point represents the average of 15 individual measurements of the solution. The range of measured values for each data point is shown as the error bars. Additional data regarding the reproducibility of the measurements and the absence of sample carry-over is given in the following sections.

## Reproducibility

To measure the reproducibility of DNA measurements, fifteen measurements were made on each of the DNA solutions using the nanoCell accessory. For each measurement, 1.5 μL of sample was loaded on the sample area of the nanoCell accessory. A pipette was used to remove the measured sample from the nanoCell sample area, the sample area cleaned with a Kimwipe, and a fresh sample solution was loaded in the sample area. The reproducibility results are given in Table 2 on the following page. The very small deviation and the narrow range of the measurements illustrates not only the ruggedness and robustness of the instrument, but also the ease of sampling and precision of the measurements made using the nanoCell accessory.

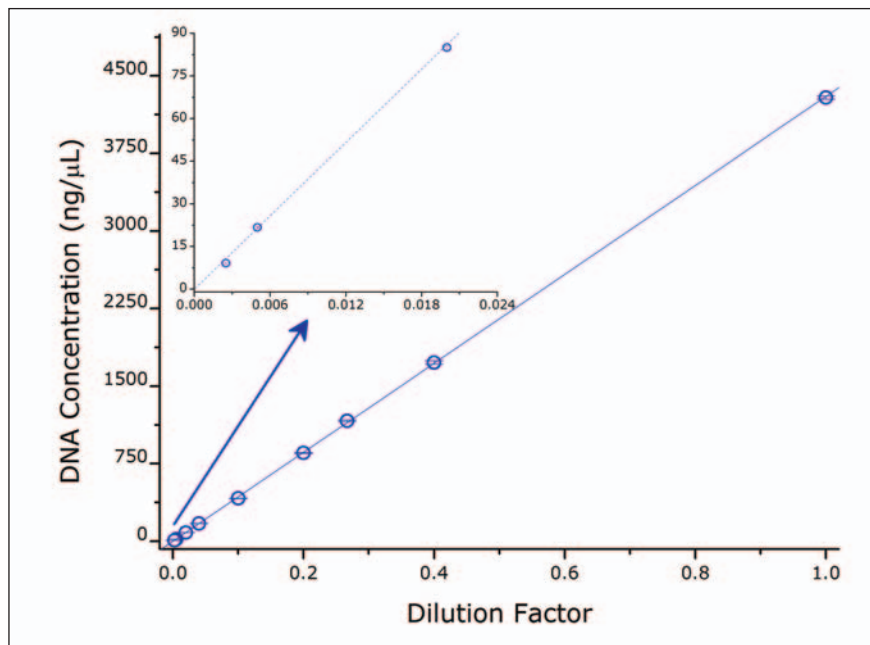


Figure 1: Linearity of DNA serial dilutions. The inset graph shows the data for the last four solutions measured. Each data point represents the average of 15 individual measurements; the range of measured values is given as the error bars (horizontal lines which lie inside the data points). All measurements were made using a 1.0 second integration time. The linear correlation coefficient is 0.99996.

Solution	Dilution Factor	DNA Concentration (ng/μL)	Standard Deviation	Measurement Range (± mean) (ng/μL)
1	1.0	4289.52	10.77	16.50
2	0.8	1743.54	7.56	16.22
3	0.4	1165.00	3.25	6.55
4	0.2	853.68	2.72	4.97
5	0.1	414.81	0.81	1.65
6	0.04	171.57	0.31	0.63
7	0.02	79.82	0.29	0.66
8	0.005	21.70	0.20	0.47
9	0.0025	9.12	0.15	0.33

Table 2: Reproducibility results for six DNA solutions of decreasing concentration. Fifteen measurements were made of each solution. The maximum deviation from the mean is shown as the measurement range.

### 260/280 Ratio

The accuracy of the 260/280 ratio, an indicator of DNA purity, was evaluated by monitoring this ratio for each solution. The results of the experiment are shown in Figure 2. Each data point in the figure represents the average of 15 individual measurements.

The range of 260/280 ratios measured with the nanoCell accessory is 0.046. This remarkably small difference illustrates the ability of the nanoCell accessory with Evolution 300 to accurately determine the absorbance at 280 nm. This measurement occurs on a sloping portion of the DNA solution spectrum. As expected, the accuracy of the 260/280 ratio measurement is not affected by dramatically decreasing the concentration of the DNA solution.

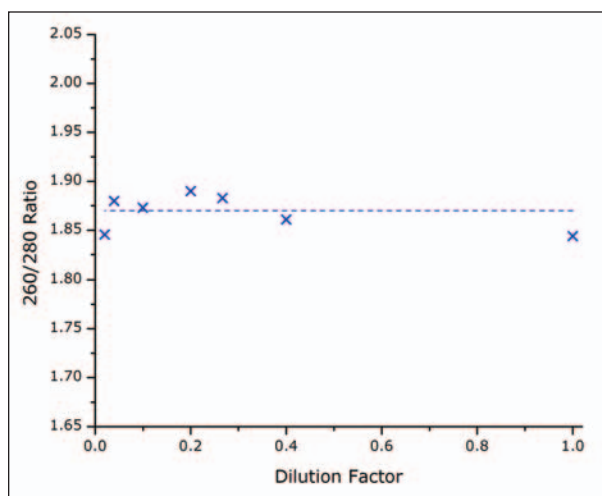


Figure 2: The 260/280 ratio of serially diluted DNA solutions

### Sample Carry-over

In order to demonstrate the sample carry-over performance of the nanoCell, three sets of five measurements were taken using a concentrated (sample 1) and diluted (sample 2) DNA solution. The results for this test are shown in Table 3.

The sample area of the nanoCell was cleaned only with a Kimwipe between solutions; no washing or rinsing with buffer was necessary.

Procedure	Readings	Average of Readings
Instrument zeroed on buffer, cleaned with Kimwipe Sample 1 loaded	4295.33	
	4302.78	
	4300.01	
	4300.61	
	4299.19	<b>4299.68</b>
Sample area cleaned with Kimwipe Sample 2 loaded	21.87	
	21.89	
	21.75	
	21.77	
	21.61	<b>21.78</b>
Sample area cleaned with Kimwipe Sample 1 loaded	4297.77	
	4291.38	
	4293.21	
	4292.27	
	4292.49	<b>4293.42</b>

Table 3: Sample carry-over experiment results

## Conclusions

In this application note, we have demonstrated the performance of the nanoCell accessory with the Evolution 300 spectrophotometer for analyzing small volume DNA samples. Excellent linearity was observed over a concentration range of 4 to 4,500 ng/μL. The reproducibility of DNA measurements was illustrated by taking 15 measurements on each sample. These results confirm not only the performance of the nanoCell when used with the Evolution 300, but also the consistency of the sample loading and the measurement precision of the nanoCell.

The 260/280 ratio over a wide concentration range reveals the performance of the instrument when small volume samples are analyzed in the nanoCell. The ability to accurately determine the absorbance at 280 nm, a shoulder of the DNA absorption peak, is crucial to the accurate measurement of DNA purity. The minimal variation observed over the wide concentration range is evidence of the reliable performance of the nanoCell accessory with the Evolution 300.

Most importantly, the lack of sample carry-over observed when dilute DNA solutions are loaded in the sample area after a concentrated sample is demonstrated. The results presented here illustrate the ease of small volume measurements using the nanoCell accessory on the Evolution 300 spectrophotometer. Specifically, the loading process requires no special washing or cleaning with buffer solution between samples. This allows samples of dramatically different concentration to be analyzed consecutively on the nanoCell with negligible sample carry-over.

Overall, we have demonstrated that the nanoCell accessory for the Evolution 300 provides consistent, reliable results for small volume DNA samples. We have provided proof of linearity across three orders of magnitude of concentration, superior reproducibility for multiple sample measurements, accurate evaluation of DNA purity using the 260/280 ratio, and negligible sample carry-over.

## Reference

1. Maniatis, T; Fritsch, E. F.; Sambrook, J. *Molecular Cloning: A Laboratory Handbook*, 2nd Edition; Cold Spring Harbor Laboratory Press: Plainview, NY 1989, Vol. 3.

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