

Quantitative Analysis of a λ DNA Using a One Drop Accessory

Introduction

For many biological samples, it is much more convenient, cost effective, and efficient to use microvolumes for structural and quantitative studies. The SAF-850 One Drop is a microsampling accessory that enables fluorescence measurements to be obtained with only 5 μ L of sample. The highly reproducible baseline allows for either simple spectrum measurements or quantitative analysis of multiple samples.

This application note demonstrates the use of the One Drop accessory to obtain fluorescence data and create a calibration curve of λ DNA labeled with PicoGreen®.



FP-8200
Spectrofluorometer

Keywords

FP-8200, Fluorescence, Microsampling, SAF-850 One Drop

Experimental

Measurement Conditions			
Fluorescence spectra		Calibration	
Excitation Wavelength	485 nm	Excitation Wavelength	480 nm
Excitation Bandwidth	10 nm	Emission Wavelength	523 nm
Emission Bandwidth	10 nm	Excitation Bandwidth	20 nm
Data Interval	0.5 nm	Emission Bandwidth	20 nm
Response Time	2 sec	Response Time	1 sec
Scan Speed	100 nm/min	Sensitivity	620 V
Sensitivity	700 V		



Figure 1. Flow chart illustrating the sample preparation procedure using the One Drop accessory.

Results

The emission spectra of λ DNA for a variety of concentrations is shown in Figure 2 and indicates that the maximum emission wavelength is 523 nm.

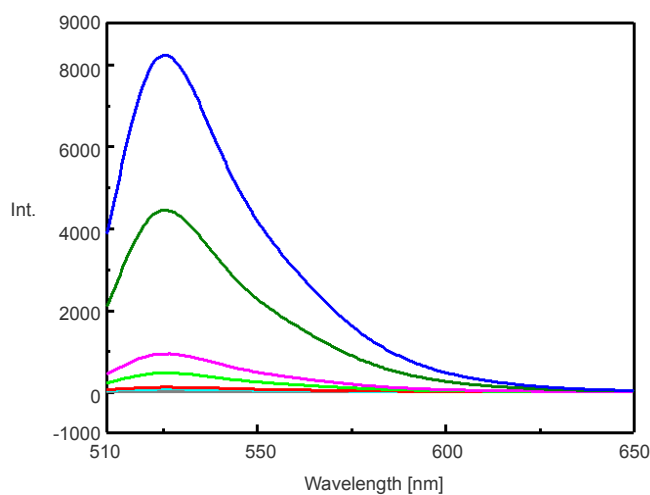


Figure 2. Emission spectra of λ DNA labeled with PicoGreen®.

The fluorescence intensity at the maximum emission wavelength (523 nm) was then measured five times for each sample to ensure measurement reproducibility. Table 1 shows these measurement reproducibility results for each sample concentration.

Table 1. Measurement reproducibility results.

Concentration [ng/mL]	1	2	3	4	5	Average	SD	CV(%)
0	53.3	52.4	55.9	53.1	55.2	54.0	1.49	2.8
1	63.6	68.1	65.9	66.5	65.1	65.8	1.68	2.6
5	110.3	106.7	105.1	104.0	110.0	107.2	2.86	2.7
10	157.6	155.5	156.1	153.0	151.7	154.8	2.39	1.5
50	447.1	465.3	460.2	455.8	469.0	459.5	8.56	1.9
100	865.9	856.9	848.3	850.6	853.9	855.1	6.86	0.8
500	3842.7	3831.0	3858.0	3828.9	3811.0	3834.3	17.42	0.5
1000	7766.2	7992.1	7925.3	7972.8	7805.7	7892.4	101.15	1.3

A calibration curve was then created using the average value for each concentration from the reproducibility results in Table 1 and is shown in Figure 3. The equation of the line fit to the calibration curve is $y = 7.780 \cdot c + 57.5211$. The correlation coefficient is 0.9999 and the standard error is 6.819, indicating a good fit.

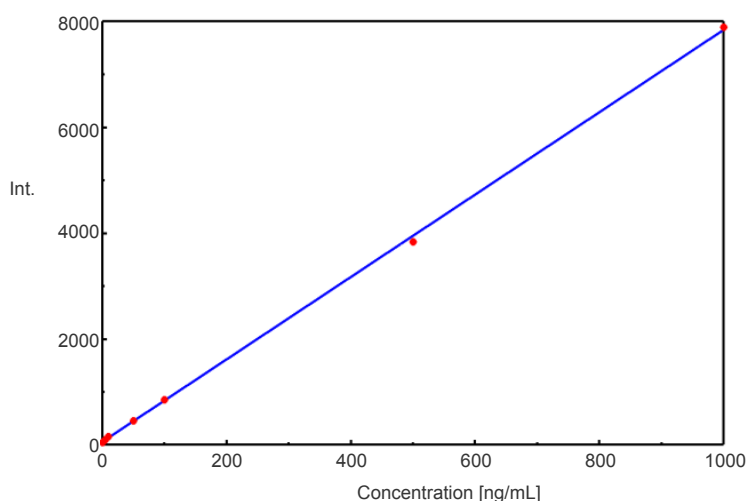


Figure 3. Calibration curve of λ DNA labeled with PicoGreen®.

Conclusion

This application note demonstrates the measurement reproducibility of the One Drop microsampling accessory by obtaining a calibration curve with good linearity over a wide concentration range.