

Quantitative analysis of λ DNA using SAF-850 One drop accessory

Introduction

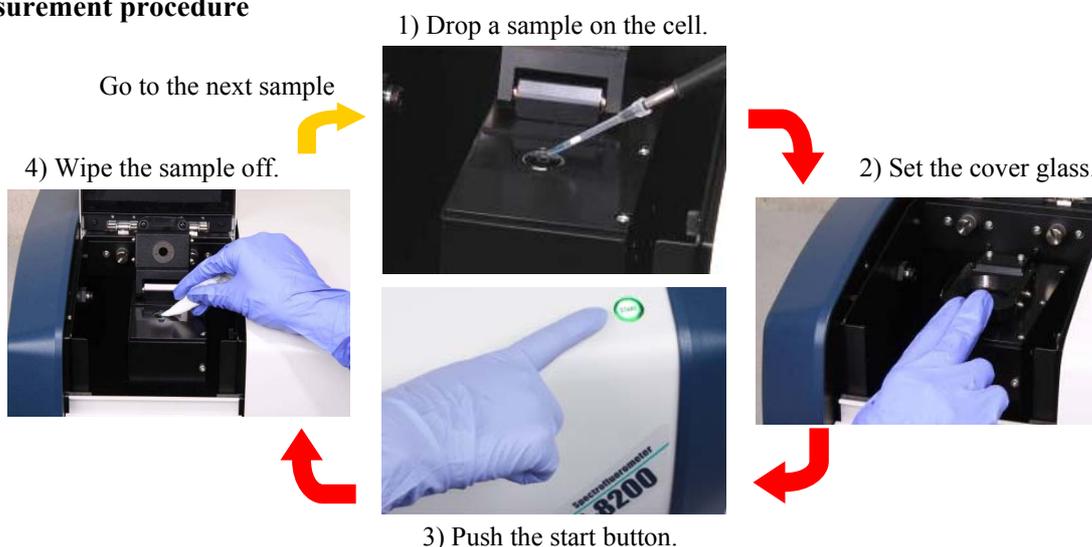
SAF-850 One Drop accessory is a special accessory, enabling fluorescence measurement by just dropping 5 μ L of the sample on a cell and then covering it with another glass. This simple way of measurement is the most suitable and effective for quantitative analysis of multiple samples such as fluorescently-labeled DNA and various kinds of fluorescent dye. In this application data, the measurement result of λ DNA labeled with PicoGreen is shown.

Keywords: One Drop, nucleic acid, reproducibility, linearity

Measurement system

FP-8200 Spectrofluorometer
SAF-850 One Drop accessory

Measurement procedure



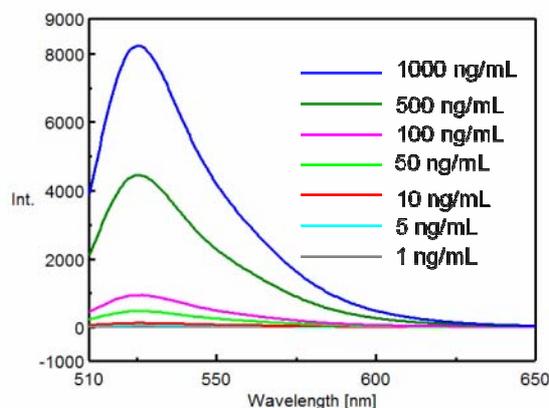
Samples

• λ DNA labeled with PicoGreen®: 0, 1, 5, 10, 50, 100, 500, 1000 ng/mL

Results

1) Spectra

The emission spectra of sample with each concentration with Ex. Wavelength of 480 nm are shown in Figure 1. The result indicates that the peak wavelength is 523 nm.



[Measurement conditions]

Measurement mode:	Emission
Ex wavelength:	485 nm
Measurement range:	510-650 nm
Ex bandwidth:	10 nm
Em bandwidth:	10 nm
Scanning speed:	100 nm/min
Data interval:	0.5 nm
Response:	2 sec
Sensitivity:	700V

Figure 1. Emission spectra of λ DNA labeled with PicoGreen®

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2) Measurement reproducibility

Table 1 shows the result of 5 times measurements of the sample with each concentration.

Table 1. Measurement reproducibility

Conc. [ng/mL]	1	2	3	4	5	Ave	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

[Measurement conditions]

Measurement mode:	Emission		
Ex wavelength:	480 nm	Em wavelength:	523 nm
Ex bandwidth:	20 nm	Em bandwidth:	20 nm
Response:	1 sec	Sensitivity:	620 V

3) Linearity

The calibration curve was generated using the average value of the results in Table 1. Figure 2 shows the good linearity over a wide concentration range from 1ng/mL to 1000 ng/mL.

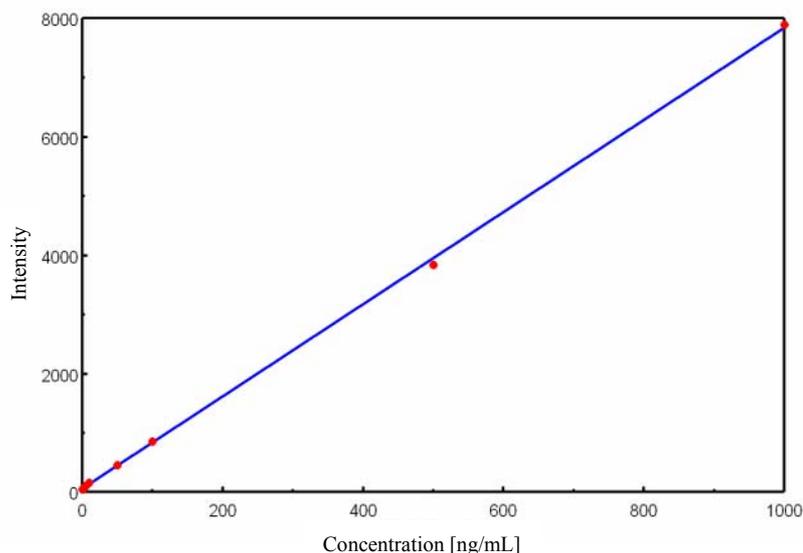


Figure 2. Calibration curve of λDNA labeled with PicoGreen®

Calibration curve equation : $Int. = 7.780 \times Conc. + 57.5211$

Correlation coefficient : 0.9999

Standard error : 6.819