

Fluorimetric Determination of dsDNA Concentrations *via* 2-point Calibration

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Introduction

Nucleic acid concentrations are typically determined with the help of absorbance measurements at 260 nm. This method is quick and easy; however, in the case of low concentrations or contaminated samples, it will reach its limits with regard to accuracy and specificity. More accurate results will be obtained with indirect measurements of fluorescence. The disadvantage of this method is the preparation, since a standard curve must be generated for each series of measurements.

To simplify this process, the Eppendorf BioSpectrometer[®] fluorescence offers “short” methods: determination of concentration is achieved by using 2 defined standards, which is equivalent to a 2-point calibration.

The method “PicoGreen[®] short” will serve as an example to illustrate this procedure, which is capable of measuring dsDNA samples quickly and easily using the fluorescent dye PicoGreen. The accuracy of this method is

comparable to the method “PicoGreen”, which employs 5 standards to determine the concentration of dsDNA (1).

One condition for the use of a 2-point calibration is strict linearity of the standard curve across more than 2 standards, which is the case for determination of dsDNA using PicoGreen.

Employing the Eppendorf UVette[®] in combination with the “PicoGreen short” method helps to save considerable amounts of reagent, since on the one hand fewer standards are required, and on the other hand the total measured volume will be minimized. The total measured volume of 2,000 µL, as described in the kit manual (please refer to the Materials section), can be reduced 20-fold, to 100 µL, by using the UVette. Thus, the number of possible measurements which can be performed with one kit will increase from 200 to 4,000.

Material and Methods

Materials

- > Eppendorf BioSpectrometer fluorescence
- > Quant-iT[™] PicoGreen dsDNA Kit
- > Eppendorf UVette
- > Water (Molecular Grade)

Method

All reagents required for determination of the concentration of dsDNA via fluorescence are included in the kit. Working solution and reagent solution are prepared in accordance with the manufacturer’s instructions. It is recommended to perform the measurements in the Eppendorf UVette, as this

will considerably reduce the amount of both working solution and reagent solution required.

Two standards are needed for the 2-point calibration:

- > Standard 1 (0 ng/mL): equivalent to the working solution without reagent. Standard 1 simultaneously serves as the blank solution.
- > Standard 2 (1,000 ng/mL): obtained by preparing a 1:100 dilution from the dsDNA solution included in the kit (100 µg/mL).

Both standards are then diluted 1:1 with reagent solution immediately prior to measurement.

The final concentrations of both standards are therefore:

- > Standard 1: 0 ng/mL
- > Standard 2: 500 ng/mL

For sample measurement, the method "PicoGreen short" is called up in the Eppendorf BioSpectrometer fluorescence (see Figure 1).

Samples are then diluted 1:1 with reagent solution in the same manner as the standards.

Following opening of the method, parameters may be verified or, if applicable, adjusted using the soft key "Edit" (Figures 2a and 2b).

The soft key "Next" will guide the user to the area "Measure Standards" within the method. Standard 1 is first measured as blank and subsequently as standard 1. Standard 2 is measured immediately thereafter. This concludes the 2-point calibration (Figure 3).

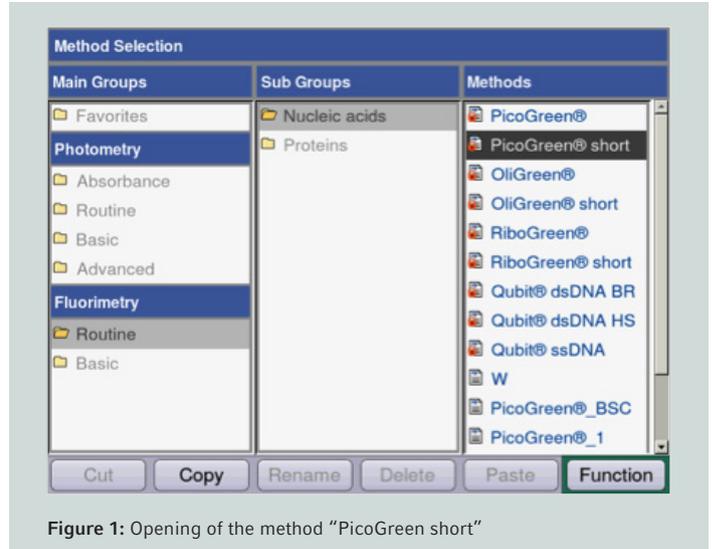


Figure 1: Opening of the method "PicoGreen short"

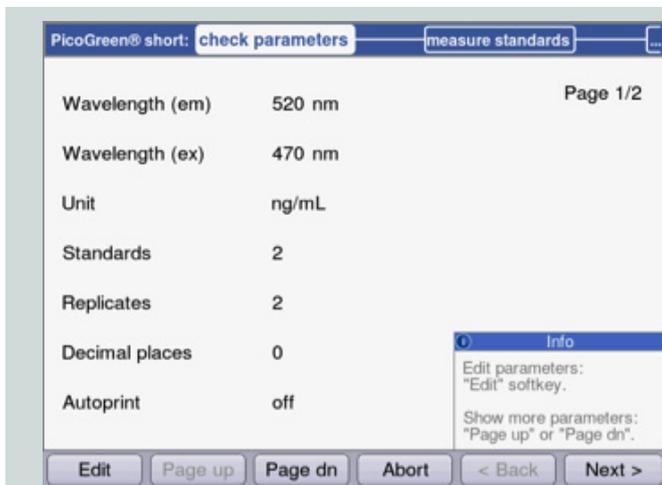


Figure 2a: Verification of measurement parameters

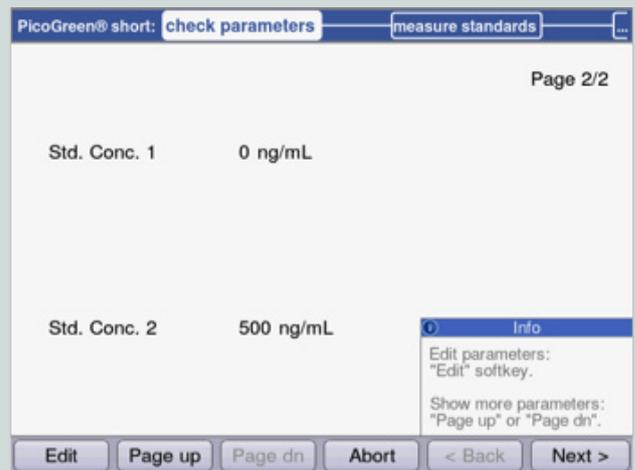


Figure 2b: Verification of standard concentrations

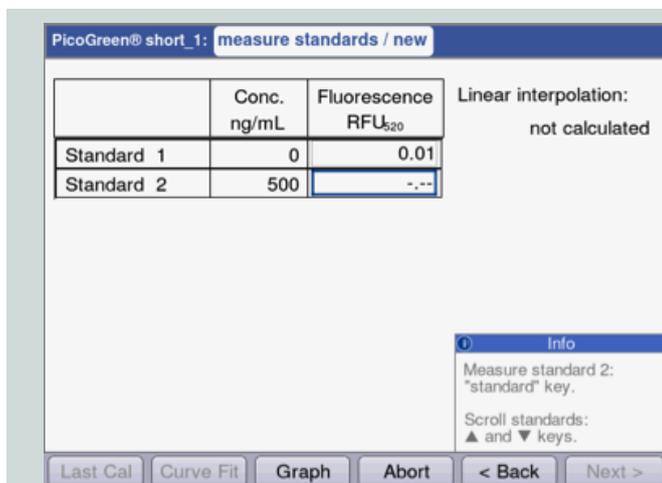
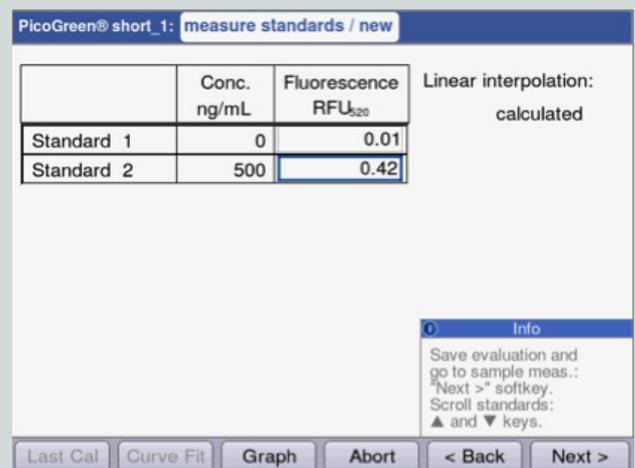


Figure 3: Individual steps of a 2-point calibration



The generated standard curve may be called up using the soft key "Graph" (Figure 4).

The soft key "Next" will take the user to the "measure sample" step of the method. The measured sample is now displayed on the standard curve (Figure 5).

Conclusion

The "PicoGreen short" method within the Eppendorf BioSpectrometer fluorescence is a quick and simple method for highly specific determination of the concentrations of dsDNA samples.

In addition, the use of the UVette enables a 20-fold increase in the number of measurements which can be performed with one reagent kit.

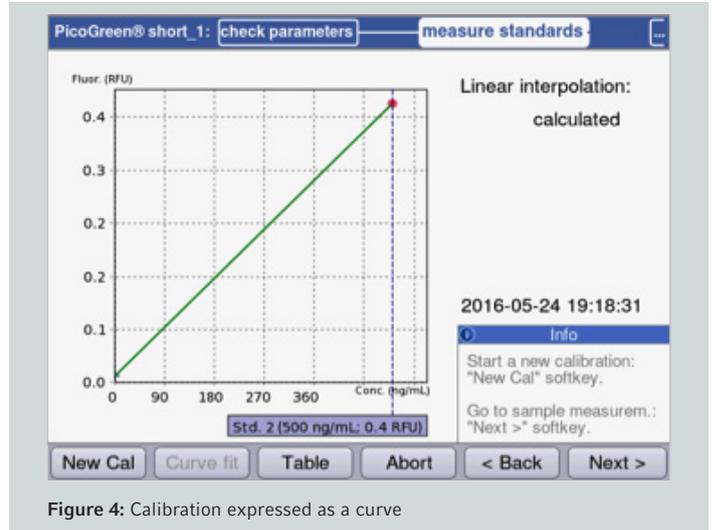


Figure 4: Calibration expressed as a curve

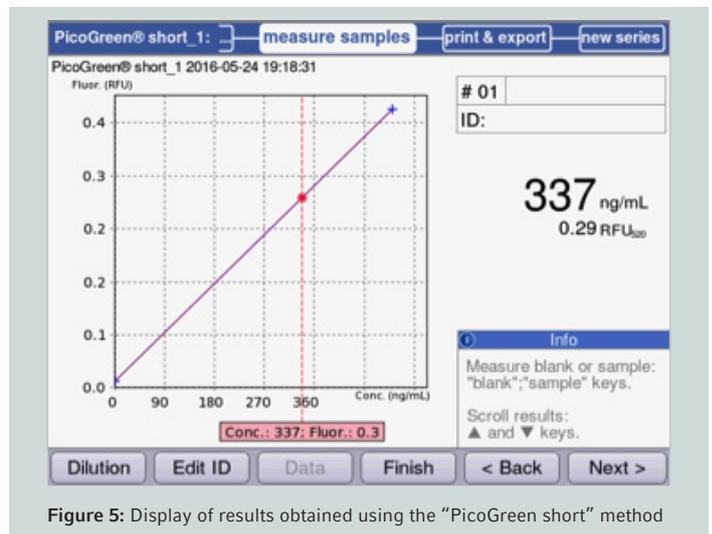


Figure 5: Display of results obtained using the "PicoGreen short" method

Literature

[1] Armbrecht, M, Gloe, J, Goemann, W. Determination of nucleic acid concentrations using fluorescent dyes in the Eppendorf BioSpectrometer® fluorescence. Eppendorf Application Note No. 271 (2013)

Ordering Information

Description	Order no. international	Order no. North America
Eppendorf BioSpectrometer® fluorescence		
230 V/50-60 Hz, electrical plug Europe*	6137 000.006	
120 V/50-60 Hz, electrical plug North America	6137 000.014	6137000014
UVette® routine pack 220 nm – 1,600 nm		
Eppendorf Quality™ purity, re-sealable box, 200 pcs	0030 106.318	952010069

* Additional electrical connection variants available

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