eppendorf

SHORT PROTOCOL No. 37

Using the Qubit™ dsDNA BR Kit on the Eppendorf BioSpectrometer® fluorescence

Martin Armbrecht, Eppendorf AG, Germany

Introduction

In addition to the Qubit dsDNA HS Kit, the Qubit dsDNA BR Kit is also very well suited for use in the BioSpectrometer fluorescence (1). Instead of an effective range of 0 - 500 ng/mL, the BR kit encompasses a range between 0 and 5000 ng/mL dsDNA. The following article will describe the experimental procedure using the UVette®.

Materials and Methods

Materials
> BioSpectrometer fluorescence,
> UVette
> Qubit dsDNA BR Assay Kit

Preparation of samples and standards:
Standards and samples are diluted 1:20 in Qubit working buffer as described in the kit protocol:
5 µL sample or standard are diluted with 95 µL of measurement buffer, respectively, directly inside the UVette (Σ=100 µL), and mixed well using a pipette. The preparation will be ready for measurement following an incubation time of 5 minutes.

In order to determine the sample concentrations using the BioSpectrometer fluorescence, it is recommended to perform the calibration using 4 standards, followed by quadratic regression analysis of the standard curve. For this purpose, two additional standards need to be prepared from Standard 2 (Component D: 100 µg/mL dsDNA) of the Qubit BR Kit. Table 1 shows an overview of the respective pipetting protocol.

Table 1: Additional standards

Concentration of the standard	**Dilution**	**Example:** 20 µL total volume	**Final concentration following 1:20 dilution** (5 µL standard + 95 µL Qubit working solution)
100 µg/mL	Standard 2 (undiluted) (Component D)	–	5000 ng/mL
50 µg/mL	50 % Standard 2 + 50% working buffer	10 µL Standard 2 + 10 µL Qubit™ dsDNA BR buffer	2500 ng/mL
20 µg/mL	20 % Standard 2 + 80% working buffer	4 µL Standard 2 + 16 µL Qubit™ dsDNA BR buffer	1000 ng/mL
0 µg/mL	Standard 1 (Component C: undiluted)	–	0 ng/mL

The protocol for the Qubit dsDNA BR Kit is largely equivalent to the protocol generated for the HS Kit (1). The Qubit™ dsDNA BR buffer is included in the kit (Component B). It is also used to prepare the Qubit working solution: the Qubit™ dsDNA BR reagent (Component A) is diluted 1:200 in the Qubit™ dsDNA BR buffer. The working solution is also required for sample measurement. Table 2 shows the volume of working solution needed for the measurement of approximately 20 samples, including standards.

Table 2: Volume of working solution required for 20 samples

Measurement in the Qubit (200 µL measurement volume)	**Measurement using UVette and BioSpectrometer fluorescence**
20 * 190 µL (samples) = 3800 µL 2 * 190 µL (standards) = 380 µL Σ = 4180 µL	20 * 95 µL (samples) = 1900 µL 4 * 95 µL (standards) = 380 µL Σ = 2280 µL

The amount of Qubit working solution (WS) that is required for X number of samples in each of the measurement systems can also be calculated using the following formula:

$$Vol_{WS} = (X*D) + (Y*D)$$

WS = working solution
X = number of samples
Y = number of standards
D = measurement system-dependent volume:
> Qubit = 190 µL
> BioSpectrometer + UVette = 95 µL

Parameter selection and measurement of standards on the BioSpectrometer fluorescence:
On the BioSpectrometer, the pre-programmed Qubit-BR method can be used (figure 1).

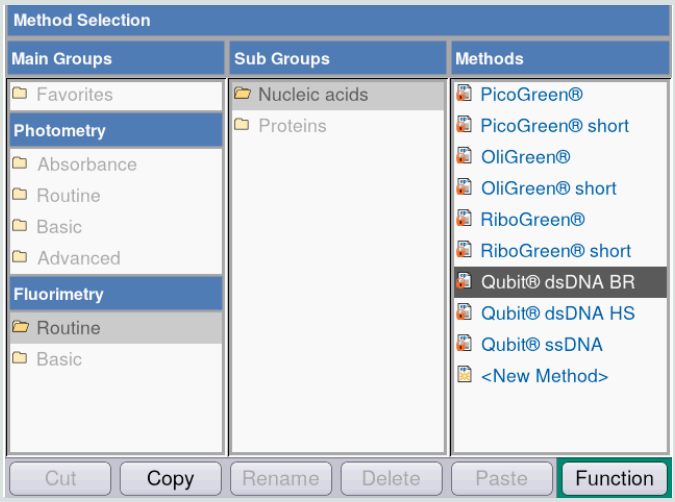
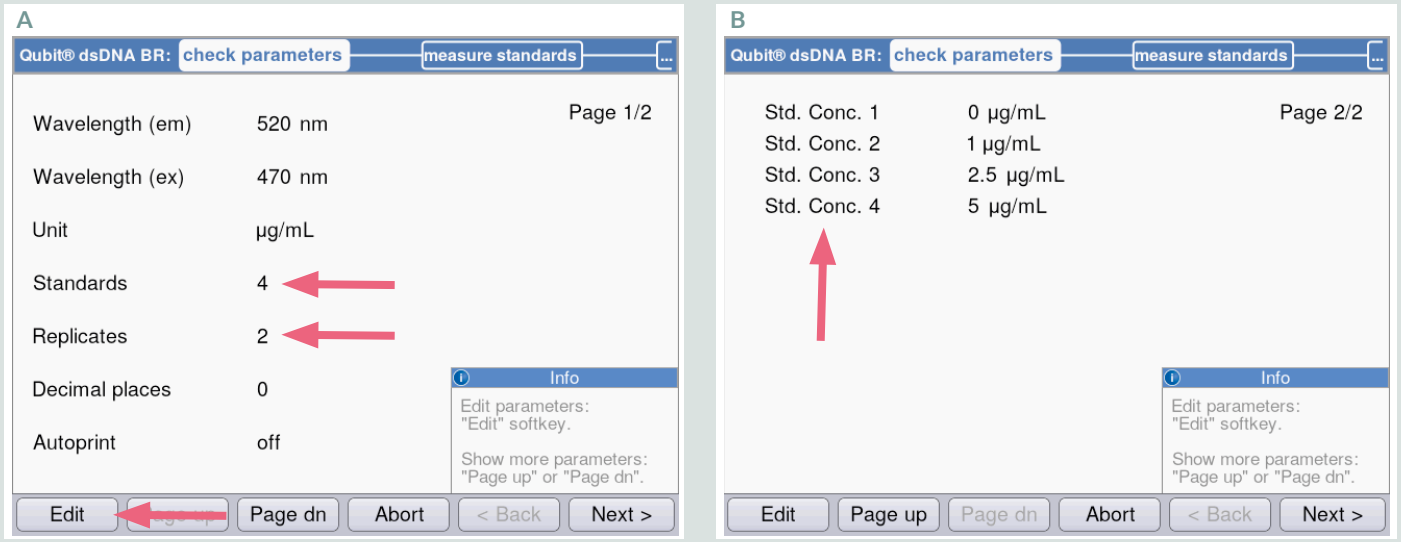


Figure 1: Selection of the pre-programmed Qubit method

The number of standards to be measured and their respective concentrations are then defined in the area "Check Parameters" prior to measurement (figures 2A and 2B).



Figures 2A and 2B: Definition of the number of standards and their respective concentrations:
A: The "Edit" function allows you to change the number of standards and replicates (red arrows).
B: The 4 standard concentrations are pre-selected but may be changed if required (red arrow).

The pre-programmed quadratic regression is recommended for standard curve analysis.
All regression analyses for standard curves may be adjusted on the BioSpectrometer via the "Curve Fit" function (figure 3).

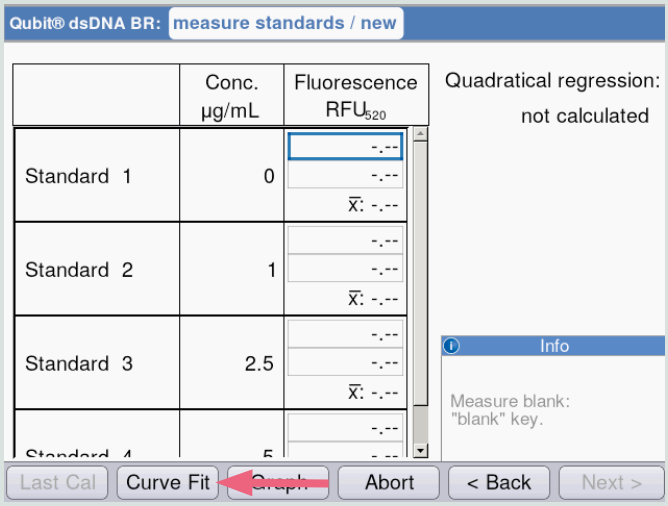


Figure 3: Fitting of the regression analysis via "Curve Fit" (red arrow).

Results

The samples are measured directly following measurement of the standards. Figure 4A depicts the example of the quadratic regression which results from the measured standards (0, 1000, 2500, 5000 ng/mL). Figure 4B shows sample measurements relative to the standards. The sample results are then displayed directly in relation to the standard curve.

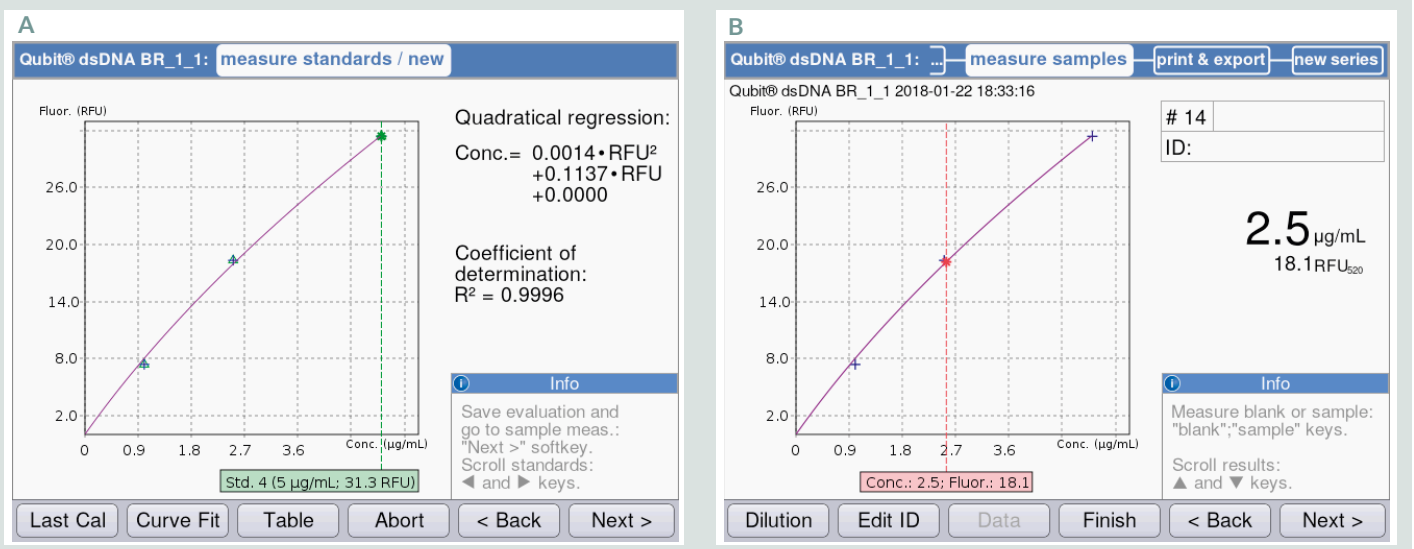


Figure 4: sample and standard measurements in the BioSpetrometer fluorescence
A: Standard curve; B: Example result

Conclusion

Besides the Quant-iT dsAssay Kit (PicoGreen®) and the Qubit dsDNA HS Kit, the Qubit dsDNA BR Assay Kit represents an additional option that allows quantification of low concentration dsDNA samples in the BioSpectrometer fluorescence with high specificity and sensitivity (1,2,3,4).
In contrast to the HS Kit (1,4), the standard curve of the BR Kit does not follow a linear regression; for this reason, quadratic regression analysis is applied (fig. 4).

As for the Qubit dsDNA HS Kit, the UVette as well as the Eppendorf µCuvette® G1.0 were tested. Since, according to the manufacturer's specifications, the reagent buffer (Component B) contains DMSO and adherence of the sample to the hydrophobic sample carrier of the µCuvette is therefore limited, the UVette is recommended for use with the BR Kit. In conclusion, the use of the UVette, in combination with the Qubit dsDNA BR Kit and the BioSpectrometer fluorescence, enables the saving of half the reagent volume as compared to the Qubit system.

Literature

[1] Armbrecht, M. Using the Qubit™ dsDNA HS Kit on the Eppendorf BioSpectrometer® fluorescence. Eppendorf Short Protocol No. 36 (2018)
[2] Armbrecht, M. Fluorimetric Determination of dsDNA Concentrations via 2-point Calibration. Eppendorf Short Protocol No. 18 (2016)
[3] 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)
[4] Armbrecht, M. Economic DNA determination in the Eppendorf BioSpectrometer® fluorescence using Qubit™ Assay kits Application Note No. 402 (2018)

Ordering information

Description	Order no. International	Order no. North America
Eppendorf BioSpectrometer® fluorescence 230 V/50-60 Hz, electrical plug Europe, additional electrical connection variants available 120 V/50-60 Hz, electrical plug North America	6137 000.006 6137 000.014	6137000014
Eppendorf µCuvette® G1.0, Microvolume measuring cell for Eppendorf BioPhotometer and BioSpectrometer	6138 000.018	6138000018
UVette® routine pack 220 nm – 1 600 nm Eppendorf Quality purity, resealable box, 200 pcs	0030 106.318	952010069

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com