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SHORT PROTOCOL No. 36

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

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Introduction

In addition to the quantification of dsDNA samples in the Eppendorf BioSpectrometer fluorescence via the fluorescent dye PicoGreen (1,2), Qubit Assay kits by Thermo Fisher Scientific may also be used for this purpose. The UVette® as well as the Eppendorf µCuvette® G1.0 are ideally suited to this task. With the UVette, regression analysis of the standard curve may be carried out using a simple 2-point calibration (linear interpolation) as described in the original Qubit protocol; however, when using the µCuvette, it is recommended to measure 4 standards and perform a quadratic regression analysis (3).

Materials and Methods

Materials

> BioSpectrometer fluorescence
> UVette, Eppendorf µCuvette
> Eppendorf Safe-Lock tubes 0.5 mL
> MixMate®
> Qubit dsDNA HS Assay kit

Preparation of samples and standards

Standards and samples are diluted 1:20 in Qubit working buffer as described in the kit protocol. The measurements can be performed in the UVette as well as in the µCuvette. Prior to measurement, samples and standards should be pre-incubated for a period of at least 5 minutes:

UVette
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. This preparation is ready to be measured.

µCuvette
2 µL sample / standard and 38 µL buffer (Σ=40 µL) are transferred to a 0.5 mL Safe-Lock tube and mixed well by vortexing (MixMate). Measurements performed in the µCuvette require only 5 µL of the preparation (3).

Determination of sample concentration in the BioSpectrometer, with the help of the UVette, may be carried out via 2-point calibration (linear interpolation) using the standards provided in the kit (final concentrations: 0 and 500 ng/mL). In this case, no changes to the protocol need to be made, except for volume adjustments. Quadratic regression analysis is recommended when working with the µCuvette, which requires a minimum of 4 standards.

The two additional standards may be generated directly from standard 2 (component D: 10 µg/mL dsDNA) of the Qubit HS kit (table 1).

Table 1: Additional standards

Standard concentration	Dilution	**Example:** 20 µL total volume	Final concentration after 1:20 dilution in Qubit working solution
10 µg/mL	Standard 2 (component D undiluted)	–	500 ng/mL
5 µg/mL	50 % Standard 2 + 50 % working buffer	10 µL Standard 2 + 10 µL Qubit™ dsDNA HS buffer	250 ng/mL
2 µg/mL	20 % Standard 2 + 80 % working buffer	4 µL Standard 2 + 16 µL Qubit™ dsDNA HS buffer	100 ng/mL
0 µg/mL	Standard 1 (component C: undiluted)	–	0 ng/mL

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

Table 2: Volume of working solution required for 20 samples using the respective measurement systems

Measurement in the Qubit (200 µL measurement volume)	**Measurement using UVette and BioSpectrometer fluorescence**	**Measurement in the µCuvette and BioSpectrometer fluorescence**
20 * 190 µL (samples) = 3800 µL	20 * 95 µL (samples) = 1900 µL	20 * 38 µL (samples) = 760 µL
2 * 190 µL (standards) = 380 µL	2 * 95 µL (standards) = 190 µL	4 * 38 µL (standards) = 152 µL
Σ = 4180 µL	Σ = 2090 µL	Σ = 912 µL

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

$$Vol._{WS} = (X*D) + (Y*D)$$

WS = working solution
X = number of samples
Y = number of standards
D = volume specific to the system:
> Qubit = 190 µL
> BioSpectrometer + UVette = 95 µL
> BioSpectrometer + µCuvette = 38 µL

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

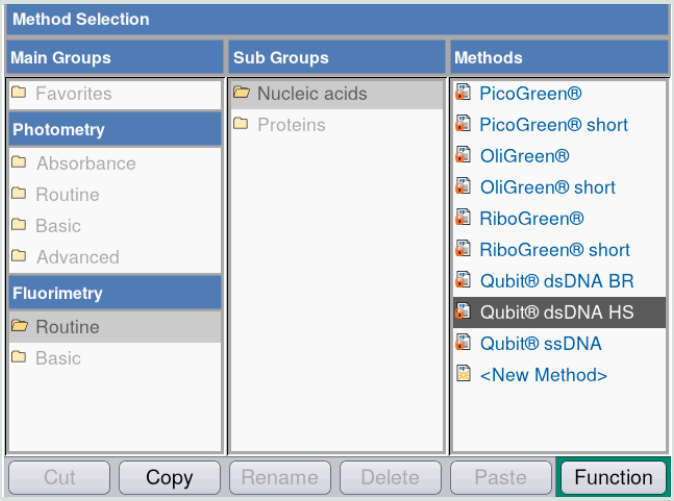
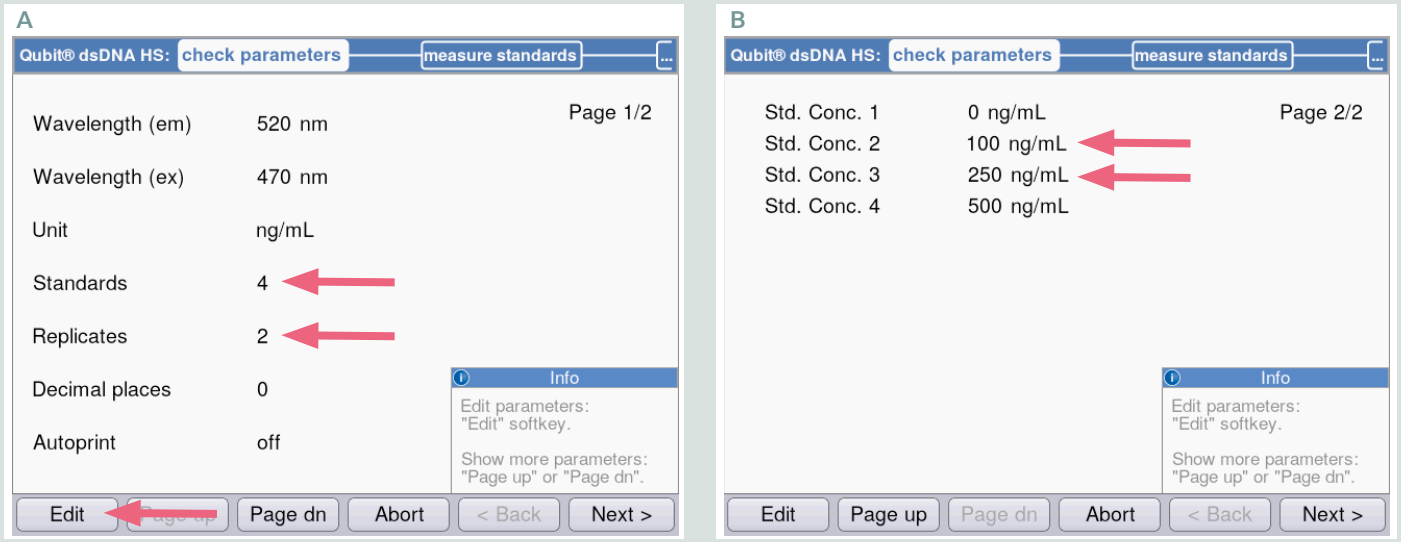


Figure 1: Selection of the pre-programmed Qubit method

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 numbers of standards and their respective concentrations:
A: The "Edit" function allows you to change the number of standards. If the µCuvette is chosen for the measurements, the 4 pre-determined standards can be used as pre-programmed in the BioSpectrometer software. With the use of the UVette the number of standards to be measured may be set to "2". The number of replicates to be measured per standard may be set to "1" (red arrows).
B: When using the µCuvette, the 4 pre-determined standard concentrations may be retained, whereas standard concentrations 2 and 3 can be omitted when using the UVette (red arrows).

Measurements using the µCuvette can take advantage of the pre-programmed quadratic regression analysis option. If standards 2 and 3 are subsequently removed when using the UVette, the standard curve analysis will automatically adjust to linear interpolation of the standards measured. All regression analyses of standard curves may be fitted on the BioSpectrometer via "Curve Fit" (figure 3).

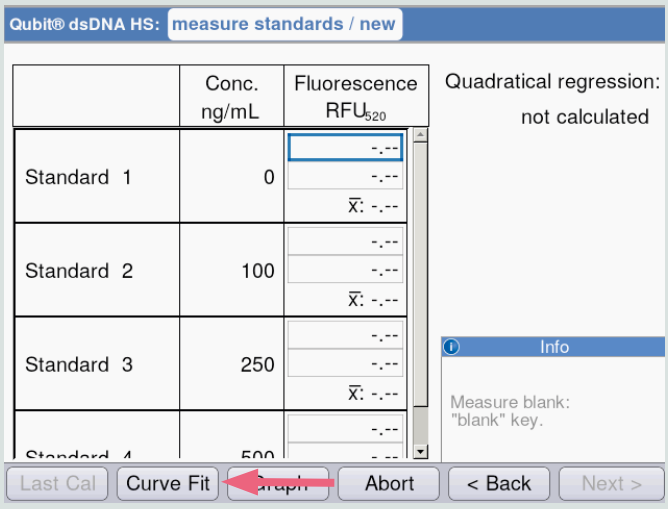


Figure 3: Fitting of the regression analysis via "Curve Fit" (red arrow).

Results

UVette – linear interpolation

The samples are measured directly following measurement of the standards. Figure 4A shows the example of linear interpolation, resulting from the two measured standards (0 and 500 ng/mL) in the UVette. Figure 4B shows sample measurements relative to the standards. The sample results are then displayed directly in relation to the standard curve.

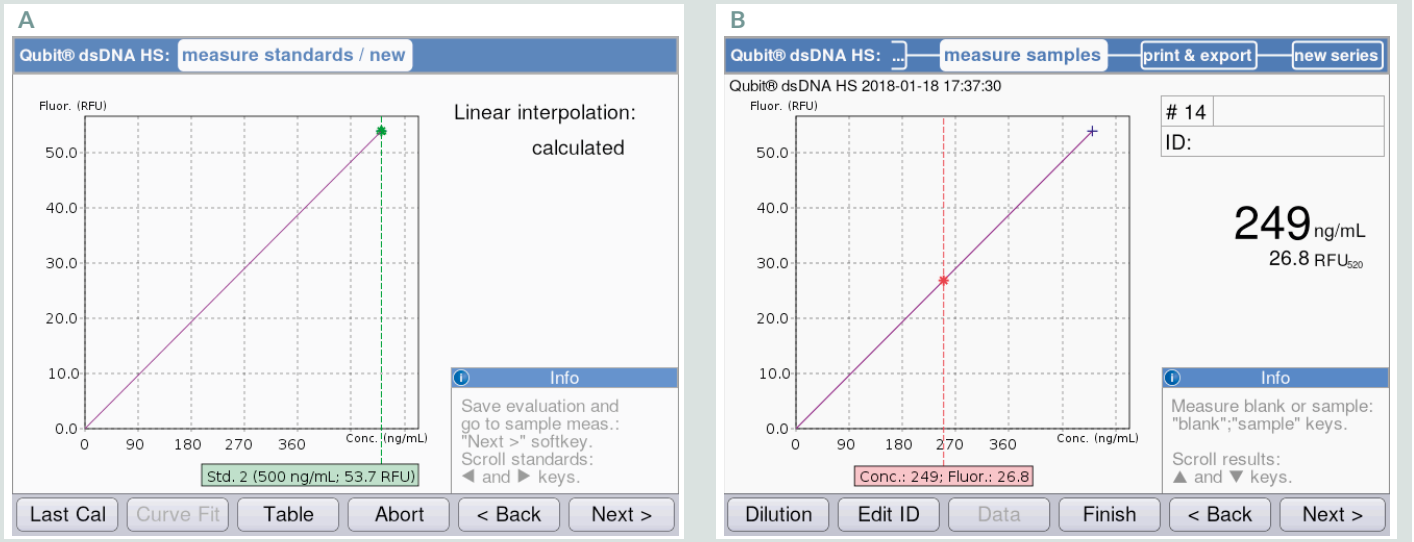


Figure 4: Measurement using the UVette, analyzed via 2-point calibration
A: Standard curve; B: Measurement result

µCuvette – quadratic regression

In contrast, quadratic regression is recommended when using the µCuvette. The result is shown in figure 5.

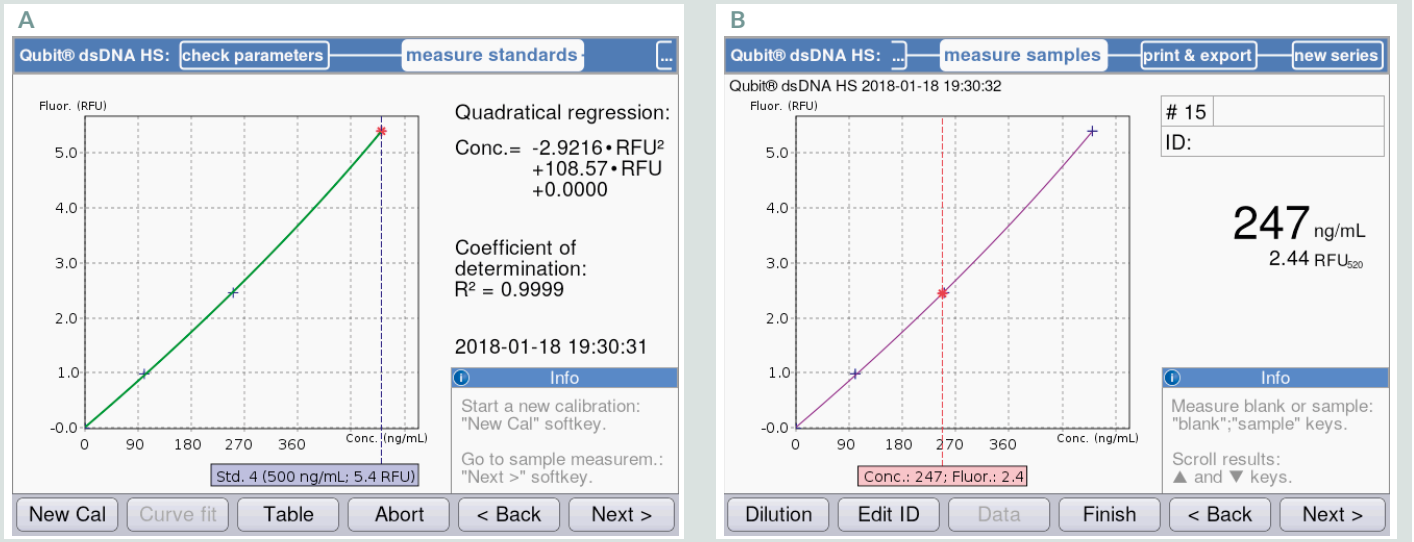


Figure 5: Measurements in the µCuvette, analyzed via quadratic regression
A: Standard curve; B: Measurement result

Depending on which cuvette is used, it is important to remember that the path length of the µCuvette is 10-fold shorter than the path length of the UVette. As a result, the µCuvette has a higher detection limit than the UVette. As a rule of thumb, it is recommended that the sample exhibit an RFU value of at least 0.5 to be detected in the BioSpectrometer fluorescence in a reproducible manner.

This value is roughly equivalent to the following dsDNA concentrations:

A UVette: approx. 5 ng/mL
B µCuvette: approx. 50 ng/mL

Conclusion

Further to the Quant-iT dsAssay kit (PicoGreen®), the Qubit dsDNA HS Assay kit offers an additional option for quantifying dsDNA samples of low concentration on the BioSpectrometer fluorescence with high specificity and sensitivity. Quantifications using the UVette require half the amount of reagent as compared to the Qubit standard protocol, and while 4 standards are recommended for measurements performed in the µCuvette, only 1/5 of the reagent volumes outlined in the original protocol are required.

Literature

[1] Armbrecht, M. Fluorimetric Determination of dsDNA Concentrations via 2-point Calibration. Eppendorf Short Protocol No. 18
[2] 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)
[3] Armbrecht, M. Economic DNA determination in the Eppendorf BioSpectrometer® fluorescence using Qubit™ Assay kits. Eppendorf 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

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