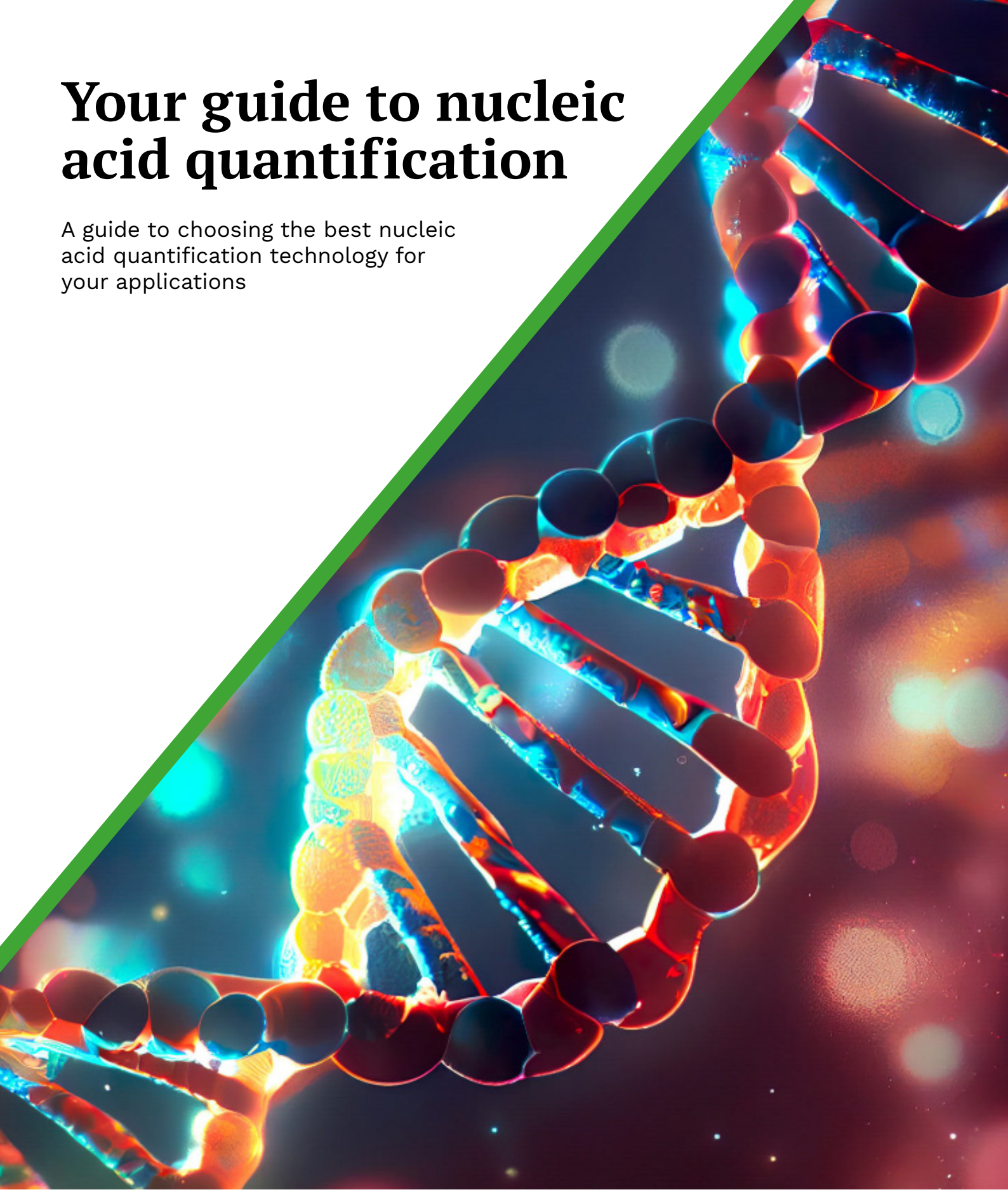
Your guide to nucleic acid quantification

A guide to choosing the best nucleic acid quantification technology for your applications



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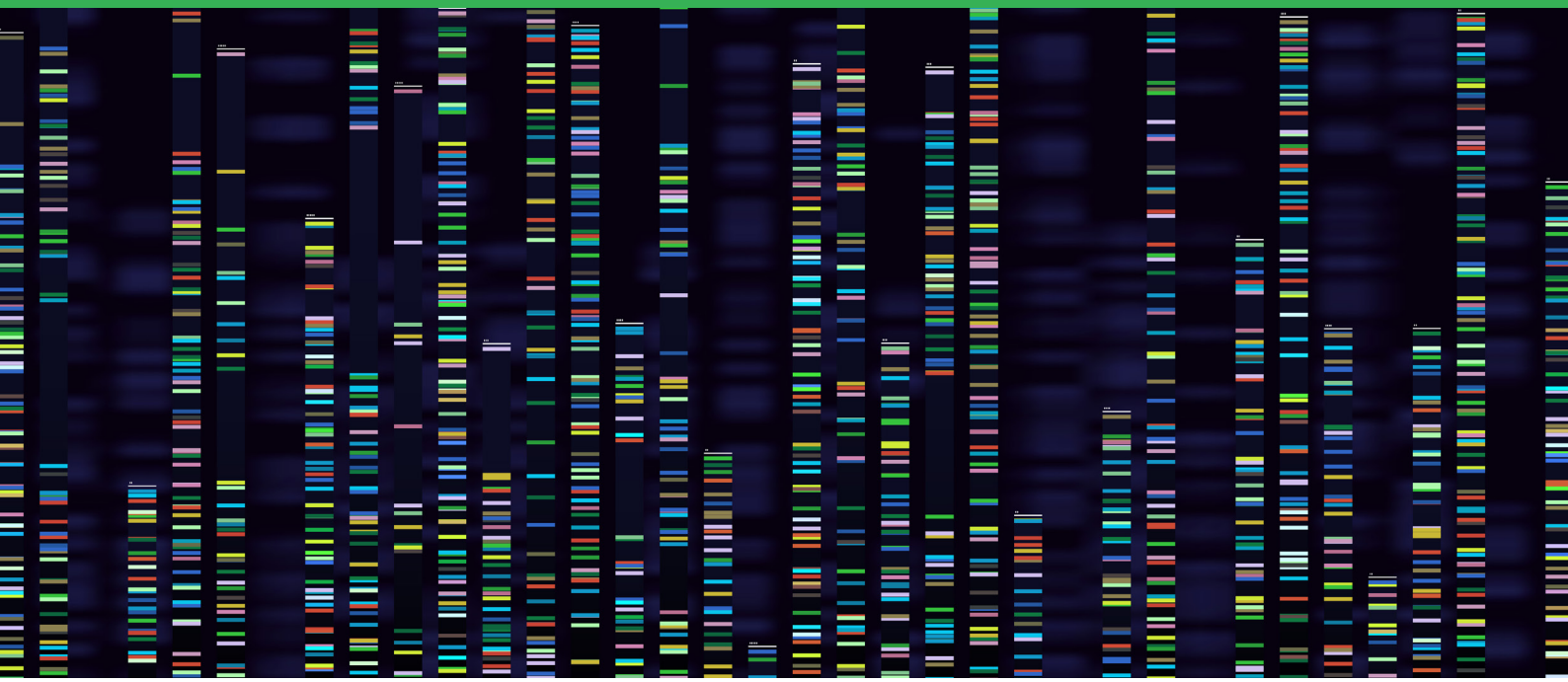

Introduction

Nucleic acid quantification is now a staple technique in genomics and molecular biology workflows, often governing the success (or otherwise) of sophisticated downstream analyses such as next-generation sequencing (NGS), polymerase chain reaction (PCR), cloning, and transfection. Whether quantifying large nucleic acids such as genomic DNA or smaller oligonucleotides like mRNA, reliable and accurate quantification upstream can be crucial to the smooth running of these more complex and expensive techniques downstream.

Two techniques have dominated the routine quantification of nucleic acids for decades – absorbance measurement at 260 nm and fluorescent dye-based methods. Many of the problems associated with early absorbance instruments have been overcome with current cutting-edge microvolume spectrophotometers, though very pure nucleic acids are still required for accurate measurement. The latest microplate readers for fluorescence measurements offer the highest ever sensitivity and specificity in quantifying nucleic acids. A wide range of technological solutions for both absorbance and fluorescence measurements are available according to specific needs and constraints. These might include sample volume, nucleic acid purity, and nucleic acid concentration range.

In this eBook, we look at Berthold Technologies' cutting-edge spectrophotometers and microplate readers through a series of case studies to help you understand when and where to employ them according to throughput requirements and the comparative

Contents

complexity of downstream applications. For example, this brochure introduces Berthold's Colibri+ family of microvolume spectrophotometers, which are ideal for low sample throughput and provide a simple and reliable solution for everyday applications. By contrast, its Tristar microplate readers are more flexible instruments offering higher throughput; these are more suited to specialized and complex downstream applications such as NGS.

Absorbance measurement

Absorbance has been the method of choice for the routine quantification of DNA and RNA for decades. To this day, it remains a simple and convenient method for rapidly measuring total nucleic acid in very small sample volumes containing medium-to-high concentrations. From these three resources, discover:

• Tips and tricks for quantifying DNA using Berthold Technologies' Colibri+ microvolume spectrophotometers. In this whitepaper, learn the six main steps involved in DNA quantification and how your choice of equipment depends upon parameters ranging from sample volume and detection limits to measurement time and wavelength range.

• How Berthold Technologies Colibri+ microvolume spectrophotometers either match or improve the performance of other manufacturers' instruments. This technical note describes how Berthold's Colibri+ instrument emerges favorably from a dsDNA test comparison of performance with the NanoDrop™ One, the DS-11, and the Nabi, demonstrating its short measurement times, broad concentration and wavelength range, and optimized sample area.

• How medium-throughput DNA quantification can be achieved on Berthold Technologies' Tristar Multimode microplate readers. This application note details how the µDrop™ Plate – with its 16 sample positions and minimum sample volume of 2 µL – combined with Berthold's Tristar 3 and Tristar 5 microplate readers offers higher-throughput DNA quantification for PCR, cloning, and sequencing applications.

Fluorescence measurement

The use of fluorescent dyes enhances both the sensitivity and versatility of DNA quantification compared with measuring the absorbance of DNA itself. Sensitivity can be up to 1000 times higher, and specific dyes can stain only specific types of nucleic acid, such as dsDNA or RNA. While larger sample volumes are required, analysis with microplate readers can achieve very high throughput using 96-well plates. From these three resources, learn:

• How fast, simple, and accurate DNA quantification can be achieved with fluorescent staining and Berthold Technologies' Tristar Multimode microplate readers. This application note demonstrates how dsDNA samples were stained with Invitrogen's Quant-iT™ PicoGreen™ dsDNA Assay Kit reagent and measured in a 96-well plate in under a minute on Tristar Multimode microplate readers. A detection limit below 0.1 pg/µL was achieved, representing a 20,000-fold sensitivity improvement over a microvolume spectrophotometer.

• How highly accurate DNA quantification for NGS applications can be achieved using the Qubit™ dsDNA BR Assay Kit adapted for use in Berthold Technologies' Tristar Multimode microplate readers. This application note describes how the dsDNA assay was adapted to 96-well format using LightCompass® software, allowing rapid quantification of 44 samples in duplicate in a single plate and demonstrating high reliability and throughput.

• How further miniaturization of the Qubit™ dsDNA BR Assay Kit on Tristar Multimode microplate readers can provide even higher throughput and reduce reagent costs with a minimal impact on quantification accuracy. This application note highlights efficiency gains by transitioning from 96-well to small volume 384-well (384sv) microplates, as the throughput increases from 44 to 187 samples per plate, while reagent consumption decreases from 200 µL to just 20 µL per sample.

INTUITIVE & RAPID MICROVOLUME ANALYSIS OF DNA, RNA & PROTEINS

The Colibri+ Microvolume Spectrophotometer

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GET CONFIDENCE IN THE QUALITY OF YOUR SAMPLE

Accurate DNA, RNA & protein quantification in less than 3 seconds

The Colibri+ system family takes microvolume quantification of DNA, RNA and protein samples to a whole new level. The system has been designed to help you accelerate your research by combining ultra-fast measurement in less than 3 seconds with intuitive 7-inch touchscreen stand-alone operation. Get confidence in the quality of your sample with reliable UV/VIS-spectroscopy from as low as 1 µl of sample and a CV of absorbance < 1%.

Whether you need microvolume measurement capability only, or the ability to perform measurements in cuvettes, the Colibri+ family offers you both options. The Colibri+ C provides a complete solution by integrating both fast microvolume measurement capabilities and a cuvette option. For laboratories that only require microvolume measurement capabilities, the Colibri+ standard model offers an affordable alternative.

DESIGNED TO SUPPORT YOUR RESEARCH

Whether you are performing real-time PCR, sequencing/ NGS, microarray or cloning experiments, the innovative features of the Colibri+ will help you to advance your application.

Colibri+ benefits at a glance

- **Fast measurement:** read your samples in less than 3 seconds.
- **Reliable results:** CV of absorbance < 1%.
- **Broad detection range:** 2 – 20,000 ng/µl for ds DNA and 0.06 – 600 mg/ml for BSA, saving you precious time by avoiding manual dilution errors.
- **Stand-alone operation:** 32 GB onboard memory, no computer required.
- **Intuitive operation:** 7" colour touchscreen and a wide variety of preprogrammed protocols simplify operation of the system.
- **Easy pipetting of samples:** Sample Guide Light enables accurate pipetting, even in poor light conditions.
- **Cuvette option (Colibri+ C model only):** with built-in 37- 45°C temperature control and stirring functions to meet specific applications. Perform kinetics, microarray and labeled protein analysis.

Easy pipetting of the sample

- **Sample Guide Light:** Your measurement starts with applying the sample. The innovative Sample Guide Light simplifies pipetting, even in poor light conditions (figure 1).

Figure 1: The Sample Guide Light enables easy sample application and helps you to detect even the smallest air bubbles. As a result, the quality of the measurement is significantly improved.

Accurate and reproducible measurements

- **Hydrophobic coating of sample window:**
  The hydrophobic coating of the sample window supports the formation of a measuring column (figure 2) from the sample liquid after closing the detector arm.
- **Shock-absorbing detector arm:**
  The shock-absorbing detector arm reduces shocks during its closure. This enables accurate measurement of samples even as little as 1 µl with a CV of absorbance < 1%.
- **Broad detection range:**
  2 – 20,000 ng/µl for ds DNA and 0.06 – 600 mg/ml for BSA, saving you precious time by avoiding manual pipetting errors.

Know the quality of your sample

- **Preprogrammed applications:**
  Poor sample quality can lead to poor results in subsequent workflows like real-time PCR quantification or NGS. The Colibri+ simplifies identification of contaminations in your samples by providing a wide variety of preprogrammed protocols for nucleic acids with QC ratios, proteins with QC ratios as well as further life science applications like protein assays (BCA, Bradford and Lowry) and cell culture OD_{600} measurements.
- **UV-VIS spectral measurement:**
  The Colibri+ provides a wide spectral range from 190 – 1,000 nm. This provides greater flexibility in the creation of custom protocols.
- **Flexible pathlength:**
  Users can choose between the two path length options 0.5 mm and 0.05 mm. This enables measurement of highly concentrated samples without dilution.

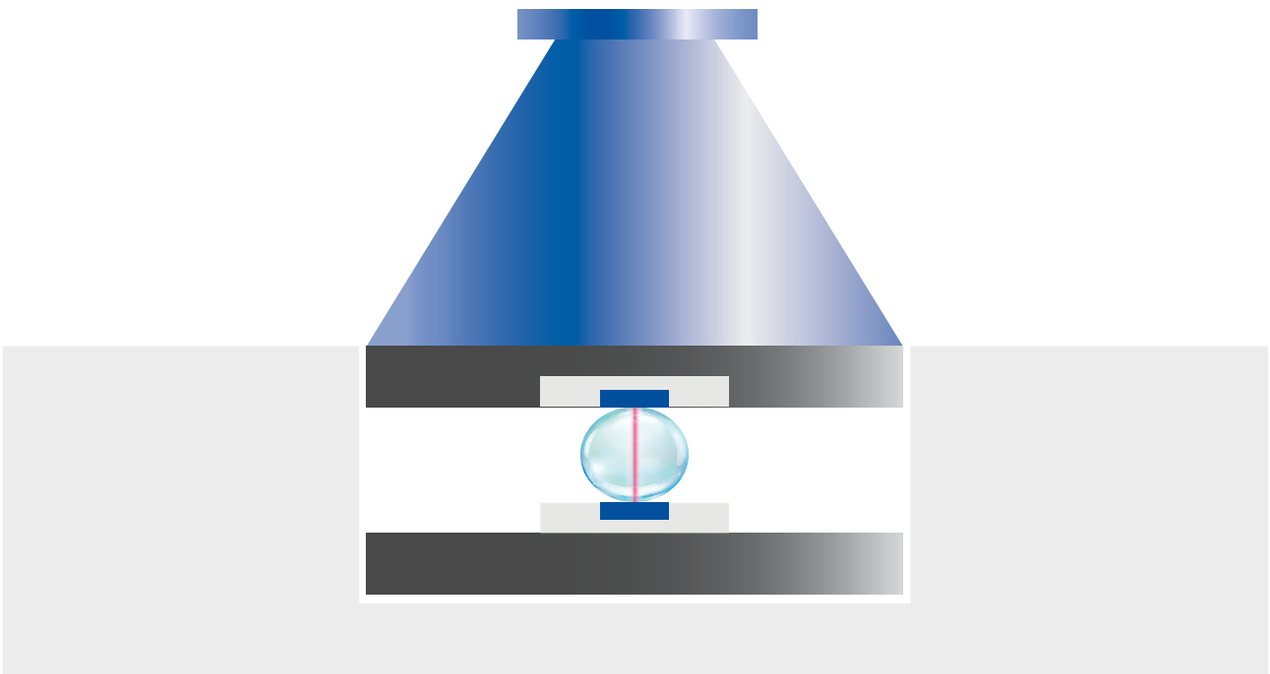

Figure 2: Hydrophobic coating of the sample window forms a measuring column when the shock-absorbing detection arm is closed. This supports the measurement of even highly concentrated samples and helps to avoid broken sample columns.

INTUITIVE OPERATION AT YOUR FINGERTIPS

Figure 3: Intuitive touchscreen operation and a wide variety of preprogrammed protocols simplify operation of the system.

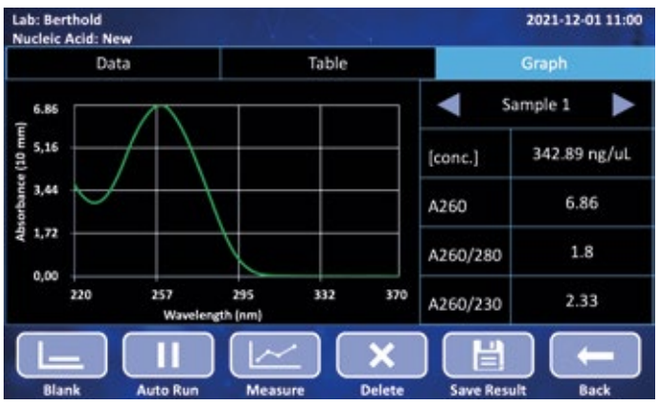

Figure 4: The graphical display offers easy and precise evaluation of your sample's quality.

Intuitive operation

The Colibri+ doesn't require a computer, saving precious lab space in combination with its small footprint of just 206 × 333 × 166 mm (W × D × H).

It is equipped with 32 GB of onboard memory. More than 500 user folders can be created, which can be password protected.

The intuitive 7" colour touchscreen operation enables you to analyze your samples with the touch of a button (figures 3 and 4).

The Colibri+ C model can be conveniently operated as a stand-alone device and can also be connected to a PC, e.g. to easily create reports.

COUNT ON OUR SUPPORT

Support

For more than 70 years, Berthold Technologies has been providing customers with life science system solutions. We understand that your applications are as individual as you are. Our dedicated technical specialists can work with you in partnership to overcome the unique challenges your application brings.

Contact our team to discuss

- Your assay or expermient design
- Data analysis questions
- Troubleshooting

Service

Berthold Expert Services provide a team of dedicated and factory-trained engineers and experts to optimize your productivity.

We and our local partners are always at your disposal.

Berthold Expert Services

- Maintenance & repair services
- IQ / OQ Services

HOW TO REACH US

Go to Contact us - Berthold Technologies GmbH & Co. KG to find your local support or technical support team.

For product FAQs, technical information, tips & tricks, go to Knowledge Base Bioanalytics - Berthold Technologies GmbH & Co. KG.


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TECHNICAL SPECIFICATIONS

System Performance (Microvolume mode)	
Sample Volume	≥ 1 µL
Detection Range dsDNA	2 – 20,000 ng/µL
Detection Range BSA	0.06 – 600 mg/ml
Path Length	0.5 mm and 0.05 mm
Measurement Time	< 3 sec
Display	7'' glove compatible colour LCD touchscreen
Optical Specifications	
Light Source	Pulsed Xenon flash lamp
Detector	2,048 CMOS
Wavelength Range	190 – 1,000 nm
Wavelength Accuracy	1 nm
Bandwidth	1.3 nm
Absorbance Accuracy	3.0% (at 0.75 A at 300 nm)
Absorbance Range (10 mm equivalent)	0.04 – 400 A
Spectral Resolution	1.5 nm (FWHM at Hg 253.7 nm)
Detection Area Material Of Construction	Stainless steel & quartz window with hydrophobic coating
General Specifications	
Dimensions (W x D x H)	206 x 333 x 166 mm
Weight	3.5 kg (7.8 lb)
Power	Input: AC 100 – 240 V, 50/60 Hz Output: DC 24 V, 2.08 A
Certifications	CE, RoHS
Applications	both models: Nucleic Acid, Protein A280, Protein Assay, OD600, Standard Curve, UV-Vis, Factor Colibri+ C only: Kinetics, Labeled Protein, Microarray

Cuvette specifications	
Cuvette type	10, 5, 2, 1, 0.5, 0.2, 0.125, 0.1 mm quartz glass or plastic cuvettes
Measurement range	0.002 ~ 1.5 A
Dimensions	Width: 12.5 mm Length: 12.5 mm Height: 45 mm Beam height: 8.5 mm
Functions	Heater (37 ~ 45 °C) Stirring (1 ~ 8 speed corresponding to 150 ~ 850 rpm)
Detection range	dsDNA: 0.3 - 75 ng/µL; BSA: 0.003 - 2.25 mg/mL
Software & Connectivity	
Connectivity	both models: USB Type A front port Colibri+ C only: additional USB Type B port for PC-connectivity
PC Software (Colibri+ C only):	PC requirements: Windows® 7 and 10, 64 bit

ORDERING INFORMATION

Models	
Colibri+ Microvolume Spectrophotometer	73179
Colibri+ C Microvolume/ Cuvette Spectrophotometer	73179-10

TRANSFORMING SCIENCE INTO SOLUTIONS

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Berthold Technologies is a global technology leader in life sciences. Our extensive range of analytical system solutions made in Germany has been trusted by scientists since 1949. These range from small standalone readers, such as microvolume spectrophotometer and luminometers to various dedicated and multimode readers, microplate washers, microplate workstations, RIA and ELISA automation products to high-end imaging systems, HPLC radio detectors and gamma-counters. It is our mission to create a healthier world, a safer environment and more efficient manufacturing processes.

Berthold Technologies GmbH & Co. KG

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Colibri+ · Id.-Nr. 73179PR2 · Rev. 02

Whitepaper

DNA QUANTIFICATION TIPS & TRICKS

Using a microvolume spectrophotometer

Introduction

DNA quantification is an important pre-analytical method, which is of great importance for many molecular biological analysis methods and can even determine their success. It is also a routine technique in procedures for translational research such as Next-Generation Sequencing (NGS), Polymerase Chain Reaction (PCR) or Real-Time PCR (quantitative PCR; qPCR), cloning or transfection, which initiates the subsequent workflow.

Absorbance has been the method of choice for routine quantification of DNA and RNA since decades. It is simple and convenient to use as no further sample treatment (other than DNA extraction) or reaction with other substances is required. However, it is not very specific (it measures all nucleic acids as a whole) and it is sensitive to contaminants, so it demands very pure DNA to be accurate. The use of fluorescent dyes permits the quantification of DNA with higher sensitivity and specificity, but fluorescence-based quantification of DNA is out of the scope of this whitepaper.

Francesc Felipe

Berthold Technologies GmbH
https://www.berthold.com/bio

Absorbance of DNA samples at 260 nm is currently most often measured using a microvolume spectrophotometer (see below), but it is also possible to use a cuvette spectrophotometer or a microplate reader. In this method, the concentration of a substance is calculated according to the Lambert-Beer law based on its absorbance. The following formula can be obtained from the original formulation of the law:

$$A = \varepsilon \cdot b \cdot c$$

Where A = absorbance at a given wavelength, ε = extinction coefficient, b = pathlength of the spectrophotometer, c = concentration of the sample.

Hence, for a pathlength of 1 cm, the concentration is equal to the absorbance at 260 nm (the absorption peak of nucleic acids), divided by the extinction coefficient.

dsDNA has an extinction coefficient of 0.02 (µg/mL)-1 cm-1, hence:

$$c_{dsDNA} = \frac{A}{0.02\ (\mu g/mL)^{-1} \cdot cm^{-1}} = A \times 50\ \mu g/mL\ (or\ ng/\mu L)$$

The same formula can be used with the respective extinction coefficients for ssDNA (absorbance x 37 µg/mL) and ssRNA (absorbance x 40 µg/mL). However, it is important to note that the formula is only valid for large nucleic acid molecules with a similar proportion of all nucleotides, such as genomic DNA, plasmids, etc. For oligonucleotides and other short nucleic acid molecules such as miRNAs, the extinction coefficient has to be calculated from the sequence of the oligonucleotide.

Workflow

DNA quantification involves 6 main steps:

1. Choosing the right equipment and materials
2. Checking measurement settings
3. Pipetting the sample
4. Performing the measurement
5. Assessing sample quality
6. Cleaning sample area

Each step has some influence on the reliability of the quantification and is hence important to performed it correctly to ensure the best results.

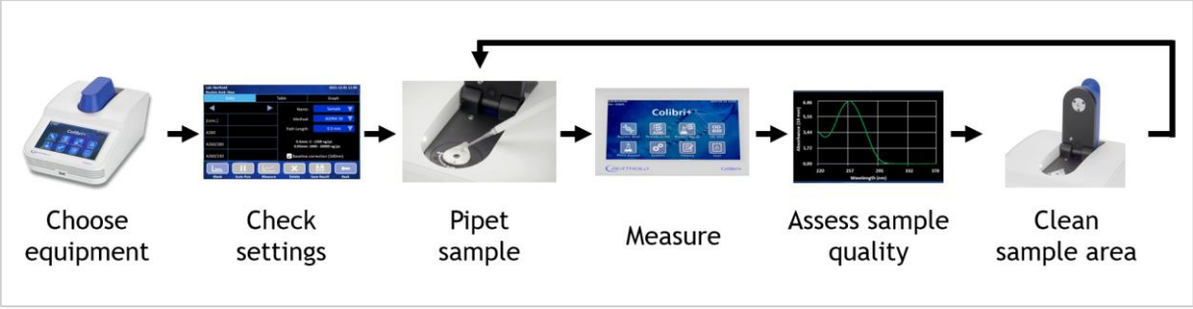

***Figure 1**: graphical representation of the workflow in a typical DNA quantification.*

1. Choosing the right equipment

If the laboratory has only recently been set up or is about to start working with nucleic acids, the first step is to select the right equipment for quantifying the DNA. This includes above all the spectrophotometer as well as the pipette and pipette tips.

1.1. Spectrophotometer

In the past, the cuvette spectrophotometer was the only available option for quantifying DNA concentration by absorption measurements. However, its application was very limited due to the large sample volume required and the small sample volume available in molecular biology procedures. Although the sensitivity of a cuvette spectrophotometer is better than that of a microvolume spectrophotometer, the required sample quantity is very large: 300-400 µL in semi-micro cuvettes and 70 µL in ultra-micro cuvettes. A real breakthrough in the application of the method was only achieved with the introduction of microvolume spectrophotometers, which allow the measurement of tiny drops of the sample (typically 1 µL). Therefore, cuvette spectrophotometers for DNA quantification have been largely abandoned, and microvolume spectrometers are the instrument of choice for single sample absorption measurements.

Definition: the area where the sample is placed is called pedestal in some instruments, in others

DESIGNED TO SUPPORT YOUR RESEARCH

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Colibri+ benefits at a glance

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Easy pipetting of the sample

- **Sample Guide Light:** Your measurement starts with applying the sample. The innovative Sample Guide Light simplifies pipetting, even in poor light conditions (figure 1).

Figure 1: The Sample Guide Light enables easy sample application and helps you to detect even the smallest air bubbles. As a result, the quality of the measurement is significantly improved.

range, the better. Most instruments can measure up to 850 nm, but some can even analyze up to 1000 nm.

- **Contamination detection:** all microvolume spectrophotometers in the market display the A_{260}/A_{230} and A_{260}/A_{280} ratios, which are a well-established method for assessing the purity of a DNA solution. Some devices also display warnings if ratios are out of a given range, so highly contaminated samples don't go unnoticed. A few instruments on the market use spectral unmixing or other mathematical algorithms instead of purity ratios to flag samples as contaminated: this has the advantage of providing further information on the nature of the contaminant, but results in low sensitivity for detecting contaminants.
- **Cuvette port:** some microvolume spectrophotometers offer the possibility to measure cuvettes in addition to microvolumes. While the cuvette port can sometimes be useful, most laboratories already have a cuvette spectrophotometer or perform colourimetric tests in microplates, which are faster and more convenient than cuvettes, and where sample volume is not critical.
- **Touchscreen operation:** touchscreen-operated microvolume spectrophotometers have become the norm, but there are still some models on the market which require a computer. The screen should be fairly large, compatible with laboratory gloves, and have a clear user interface that facilitates tapping buttons, entering sample IDs, etc.
- **User/result management:** if the spectrophotometer is going to be used by many different users, it is convenient that the software offers some type of user management, or at least different folders for different users.
- **Other features:** some microvolume spectrophotometers provide special features which are not available in most other instruments: Some allow you to measure multiple samples simultaneously, have a built-in timer or a light that makes pipetting easier and more reliable. Look for the features that are most important for your application and choose the microvolume spectrophotometer accordingly.

***Tip*:** if your samples frequently contain concentrations below 5 ng/µL dsDNA, using fluorescent dyes for quantification is more suitable for your application.

1.2. Pipette and pipette tip

Since small sample volumes (typically 1 µL) have to be pipetted in microvolume spectrophotometers, choosing the right pipette and pipette tip is of great importance. Air displacement pipettes are the most commonly used. These are recommended to pipet aqueous solutions such as purified DNA samples. Mechanical pipettes are well suited, but electronic pipettes offer greater consistency and improved ergonomics. Most 10 µL pipettes have a volume range of 1-10 µL or 0.5-10 µL. They are therefore suitable for pipetting 1 µL. However, if your microvolume spectrophotometer requires to pipet smaller volumes (e.g., to use short pathlengths), it's recommended to use a smaller pipette (0.2-2 µL or similar). Pipettes should be calibrated at regular intervals (at least once a year).

Tips used to quantify DNA should be nuclease-free and have a filter to block aerosols from the liquid sample contaminating the shaft, and subsequently contaminating later samples. Low retention tips can be beneficial to reduce the volume of sample remaining inside the tip after dispensing and help to ensure that the entire volume is dispensed.

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Tip: The pipette and pipette tip combination should be considered as a single instrument, as the same pipette may work differently when using different tips. Some pipette manufacturers even recommend recalibrating the pipette when switching to tips of a different model.

2. Checking measurement settings

Settings of a DNA quantification protocol using absorbance at 260 nm are quite straightforward: normally picking the correct DNA species (dsDNA or ssDNA) sets the conversion factor needed for the quantification, and that's it. But, depending on the software, some additional settings might be available:

- **Pathlength:** long pathlengths are used for low-concentration samples, short ones for high-concentration ones. On some instruments this is set manually, on others it is automatic, and in some you can choose between manual and automatic. Select the recommended pathlength for the expected absorbance of your samples. Be aware that the automatic mode takes longer on most devices, as the sample is measured with several pathlength settings and the optimal one is selected.
- **Baseline correction:** it corrects absorbance values for the contribution of particles in suspension (most often salt precipitates which appear when the sample is frozen). This is normally active by default at the wavelength recommended by the manufacturer (usually 320 or 340 nm), but it's always a good idea to verify that it is active.

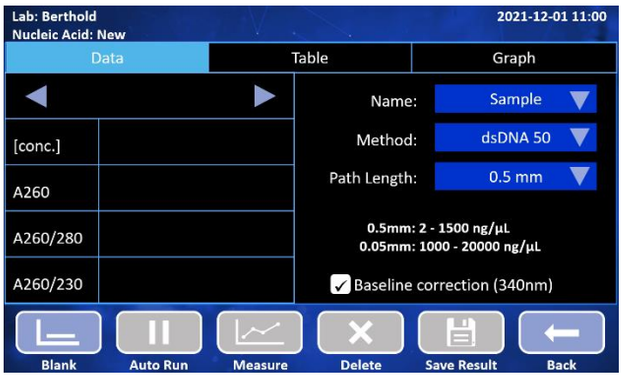

Figure 3*: typical parameters in DNA quantification*

3. Pipetting the sample

A reliable quantification starts with reliable pipetting. No matter how good the spectrophotometer is: if the wrong amount of DNA is pipetted, the wrong amount of DNA will be quantified. Thus, it is always good to keep in mind good pipetting techniques:

- Mix the sample tube gently but thoroughly, so the aliquot measured is representative of the content of the tube.
- Immersion angle of the tip should be as perpendicular to the liquid as possible, and immersion depth for small volumes should be around 1-2 mm.
- Aspiration should be careful and consistent from sample to sample.
- Some pipette manufacturers recommend forward pipetting technique to pipet small volumes, while others recommend reverse pipetting. If unsure, check the pipetting handbook of your pipette's manufacturer, or try both.
- Be careful when placing the sample: the drop has to be in the centre of the sample area of the microvolume spectrophotometer. Use one finger of your free hand to stabilize the pipet if needed.

- Ensure lighting conditions are good and allow you to clearly see the drop and sample area.
- Before closing the arm, observe if the drop is round and regular. If it flattens, the hydrophobic coating of the sample area needs reconditioning. Failing to recondition the coating of the sample area can lead to improper column formation and cross-contamination. Depending on the specific model of microvolume spectrophotometer and the way it's used, coating may need to be reconditioned often, or very seldom.
- As the drop to be measured is so small, depending on the laboratory conditions,, there may be considerable evaporation of the drop and thus concentration of the sample. If sample is exposed to air in your microvolume spectrophotometer (see above), measure as quickly as possible after placing the drop.
- Use a fresh tip for each sample.

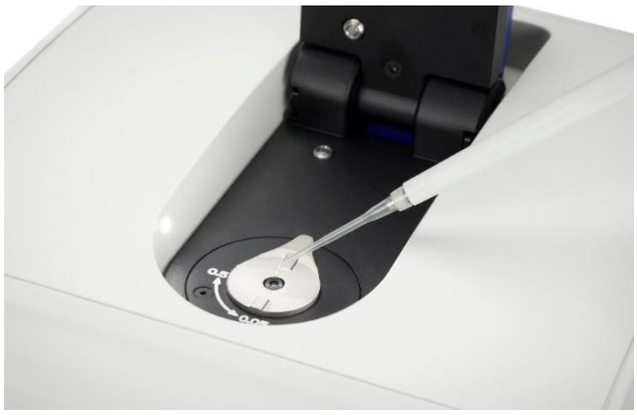

***Figure 4**: pipetting in the sample area of the Colibri+*

4. Performing the measurement

Before measuring the first sample you have to blank the instrument. Blanking means setting a reference absorbance value of zero to the buffer in which the sample is dissolved. Blanking correctly is extremely important for the reliability of the quantification: blanking with the wrong buffer or a dirty sample area will result in wrong quantification.

To perform the measurement, refer to the documentation of your microvolume spectrophotometer, as the exact blanking procedure could differ depending on its software. The typical procedure is as follows:

1. Clean the sample area with deionized or distilled water.
2. Pipette the same buffer in which the samples are dissolved (usually TE buffer for nucleic acid samples).
3. Lower the detection arm and tap the "Blank" button.
4. Wait for the measurement to complete.
5. Wipe the sample area with lint-free wipe paper.
6. Before measuring the first sample, it can be helpful to verify the blank: place a new drop of the buffer used for blanking and measure it. Check that the absorbance at 260 nm is close to zero (usually under 0.020). If it's not, clean the sample area and blank again.
7. Wipe the sample area with lint-free wipe paper.
8. Pipette the first sample.
9. Lower the detection arm and tap the "Measure" button (if necessary).
10. If the spectrum looks unusual, clean the sample area and blank again.
11. Wipe the sample area with lint-free wipe paper.
12. Continue with subsequent samples.

***Tip*: if you are measuring many samples in a row, verify the blank at regular intervals**

5. Assessing sample quality

In addition to measuring the DNA concentration of the sample, it is also important to assess its quality, as contaminated samples might be unsuitable for some downstream methods. This can be performed just after measuring each sample, or after finishing the full batch of samples. However, it is recommended to at least check the spectrum after measuring each sample, as this could reveal a bad blank that would affect all quantified samples until the instrument is re-blanked. Many of the most frequent contaminants can be estimated by measuring the absorption of the sample at wavelengths other than 260 nm: mainly 280 and 230 nm. A low ratio between the absorbance at 260 nm and the absorbance at 280 (A_{260}/A_{280}) or 230 nm (A_{260}/A_{230}) is a sign of contamination.

5.1. A_{260}/A_{280} ratio

Proteins have a higher absorption at 280 nm compared to260 nm. The ratio between the absorbances at 260 (A_{260}) and 280 nm (A_{280}) is an accepted tool for assessing protein contamination in a sample of purified DNA. The A_{260}/A_{280} ratio of a sample containing pure DNA with no protein contamination should be around 1.8, with values below 1.8 indicating contamination by protein, and higher ratios indicating contamination by **RNA**.

The sensitivity of the A_{260}/A_{280} ratio for the detection of protein contamination is low: a ratio of 1.75 (only 0.05 below the “ideal” 1.80) could already indicate a protein content of about 50% in the sample. While some systems offer a corrected DNA concentration based on the deviation of the sample's spectrum from the theoretical spectrum of pure DNA, the high amounts of protein required for the difference to be measurable make the reliability of this correction questionable.

A low A_{260}/A_{280} ratio may also be indicative of the presence of **phenol**, an additive used in some DNA purification methods.

Even small changes in the **pH** of the solution can also modify the A_{260}/A_{280} ratio by up to 0.3, downwards in acidic solutions, and upwards in basic solutions. For example, DNA samples which have been prepared with TE buffer but are diluted afterwards with water will have a more acidic pH and hence a lower A_{260}/A_{280} ratio, even if the DNA is completely pure.

A_{280} values approach the lower detection limit of the instrument faster than A_{260} values: for example, for a concentration of dsDNA of 4 ng/µL, A_{260} would be 0.080, and A_{280} for a “pure” dsDNA sample would be 0.044, and the closer to 0, the higher the variability and error of the measurement. This means that the A_{260}/A_{280} ratio is in most cases not reliable for low DNA concentrations (under 10 ng/µL). Hence, an abnormal A_{260}/A_{280} ratio may NOT indicate contamination problems if the DNA concentration is very low.

5.2. A_{260}/A_{230} ratio

Some common contaminants cause a relative increase in absorbance at 230 nm compared to 260 nm, and the A_{260}/A_{230} ratio is hence also used to assess DNA purity. The A_{260}/A_{230} ratio of pure DNA is typically between 2.0 and 2.2, with values up to 2.4 not being uncommon. A lower ratio indicates contamination by **protein, phenol, EDTA, guanidine or carbohydrates**. Hence, the presence of protein or phenol will cause a decrease in BOTH the A_{260}/A_{280} and the A_{260}/A_{230} ratios.

The information can be summarized in the following table:

Purity ratio	A_{260}/A_{280} < 1.80	A_{260}/A_{280} 1.80	A_{260}/A_{280} >1.80
A_{260}/A_{230} < 2.0	Protein Phenol	EDTA Guanidine Carbohydrates	Possible combination of RNA or high pH with other contaminants
A_{260}/A_{230} > 2.0	Low pH	**Pure DNA**	RNA High pH

***Table 1**: typical causes of deviations in purity ratios.*
Color code: orange = contaminated, green = pure DNA, white = unclear

5.3. Other quality problems visible in the spectrum

As summarized above, purity ratios are very informative about the type of contaminants present in the sample, and the presence of contaminants which alter A_{260}/A_{280} or A_{260}/A_{230} ratios is also visible in the spectrum:

- The presence of guanidine usually produces a shift of the valley, which should be visible around 230 nm, to longer wavelengths (usually around 240 nm).
- Phenol/Trizol and protein contamination also produce a shift of the valley at 230 nm, but also of the peak at 260 nm, towards longer wavelengths.
- Unusual spectra (negative values in some parts of the spectrum, ragged spectrum…) usually mean the blank was not performed properly or that the sample area was is dirty.

Some instruments perform spectral unmixing or other mathematical algorithms to the spectral information to flag samples as contaminated, as this allows sometimes to identify the contaminant, but this method is often less sensitive to detect contamination than purity ratios.

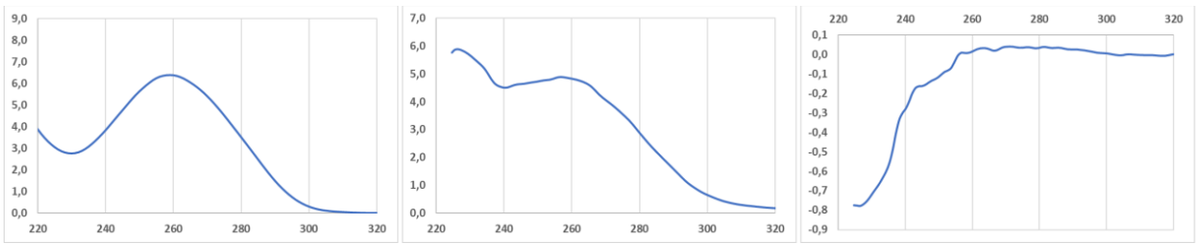

***Figure 5**: typical spectra of a high-purity DNA sample (left), a DNA sample contaminated with protein (center) and a sample measured after a wrong blanking procedure (right).*

5.4. DNA integrity

Assessing the integrity of your samples is also very important, as degraded DNA is unsuitable for many downstream methods. Unfortunately, while the spectrum of the sample measured in a microvolume spectrophotometer is very informative about the purity of the extracted nucleic acids, it does not provide any information about its integrity. This is due to the absorption spectrum of free nucleotides being identical to that of nucleotides belonging to a large DNA or RNA molecule. To assess the integrity of nucleic acids, other methods have to be used (for example, running a gel electrophoresis and visualizing the nucleic acid using an intercalating dye).

6. Cleaning sample surface

The last step is simple, but it is very important, as any contamination of the sample area could lead to an overestimation of the DNA concentration.

- Wiping the sample area with a dry lint-free wipe paper is normally enough to clean the sample surface between measurements.
- If the samples measured have high concentration, some instrument manufacturers recommend wetting the wipe paper with distilled water to ensure proper cleaning.
- Wiping the sample area with a wet lint-free wipe paper is also recommended after the last sample is measured.

Troubleshooting

Even when choosing the most suitable equipment, pipetting correctly, and following all recommendations, problems can still occur. Some frequent problems and their solutions are summarized here.

Tip: the source of many frequent problems is improper blanking. If you are getting bad results, clean thoroughly the sample area, check that you are using the correct buffer for blanking and blank again before performing any further troubleshooting steps.

Problem	Possible Cause	Solution
Measurement too high	Blanking not properly performed	Clean the sample area, check buffer used for blanking and blank again
	Surfaces of the sample area polluted	Clean reflector and sample area according to the description in the user manual
	Wrong normalization settings	Check normalization settings
	Wrong sample type selected	Select the right sample type
	Sample has air bubbles	Remove air bubbles from sample or pipet again
	Sample area not correctly closed	Open detection arm, check sample area and close detection arm
	Evaporation of sample drop increases concentration with time	Clean the sample area, pipet the sample again and measure immediately

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Problem	Possible Cause	Solution
Measurement too low	Blanking not properly performed	Clean the sample area, check that the buffer used for blanking is correct and blank again
	Wrong normalization settings	Check normalization settings
	Wrong sample type selected	Select the right sample type
	The solutions are not homogenous and well-mixed prior to sampling	Ensure all solutions are homogenous and well-mixed prior to sampling, heat up samples if needed (high concentration)
	Sample concentration too high	Dilute sample and measure again
	Wrong pipetting of the sample	Clean sample area, pipette sample and measure again
Low accuracy or reproducibility	The solutions are not homogenous and well-mixed prior to sampling	Ensure all solutions are homogenous and well-mixed prior to sampling, heat up samples if needed (high concentration)
	Wrong pathlength selected	Check that the pathlength settings are suitable for the OD of the sample and measure again
	Sample concentration too low (close to detection limit of the instrument)	Quantify using a different method (cuvette spectrophotometer, fluorescent dye)
	Sample concentration too high	Dilute sample and measure again
	Sample has air bubbles	Remove air bubbles from sample or pipet again
	Surfaces of the sample area polluted	Clean reflector and sample area according to the description in the user manual
	Wrong pipetting of the sample	Clean sample area, pipette sample and measure again
	Scratched surfaces of sample compartment	Call for service
	Instrument calibration invalid	Calibrate instrument
	Hardware malfunction (lamp, optics, alignment…)	Call for service
Low A_{260}/A_{280} ratio	Sample contaminated with protein or phenol	Troubleshoot extraction and purification procedure
	Low pH	Check that the buffer to resuspend or dilute the sample is correct
High A_{260}/A_{280} ratio	Contamination with RNA	Troubleshoot extraction and purification procedure
	High pH	Check that the buffer to resuspend or dilute the sample is correct
A_{260}/A_{280} ratio different to other spectrophotometers	Different wavelength accuracy of the compared instruments	No action required in most cases. If difference is large (<0.4), check calibration of both instruments.
Low A_{260}/A_{230} ratio	Sample contaminated with phenol, protein, EDTA, guanidine or carbohydrates	Troubleshoot extraction and purification procedure
Unusual spectrum	Sample contaminated	Troubleshoot extraction and purification procedure
	Dirty sample area	Clean thoroughly the sample area, check buffer used for blanking and blank again
	Sample used for blanking instead of buffer	Clean the sample area, check buffer used for blanking and blank again
	Sample area needs reconditioning	Recondition sample area
	Sample volume too low	Increase sample volume

Problem	Possible Cause	Solution
Sample drop flattened	Sample area needs reconditioning	Recondition sample area
Detection time too long	Pathlength set to Auto	Set a specific pathlength which is suitable for the concentration of most of your samples
	Hardware malfunction	Call for service
Other problems	Probably instrument-specific problem	Check the user manual of your microvolume spectrophotometer. Contact Technical Service if needed.

Not for use in diagnostic procedures.

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Technical Note

COMPARING MICROVOLUME SPECTROPHOTOMETERS

COLIBRI+ VS NANODROP™ ONE, DS-11 AND NABI

Abstract

The Colibri+ LB 916 Microvolume Spectrophotometer features short measurement times, broad concentration and wavelength range, as well as an optimized sample area. Its accuracy and reproducibility are comparable to the top competitors (NanoDrop™ One and DS-11), even surpassing them at some concentrations.

Introduction

Reliable and accurate quantification of DNA, RNA and proteins is needed for many downstream methods, such as qPCR, cloning, NGS, western blotting, and more. Absorbance has been the method of choice for routine quantification of DNA and RNA since decades [1]. It is simple and convenient to use, as no further sample treatment (other than DNA extraction) or reaction with other substances is required. Available sample volume is often very limited in many Molecular Biology methods, and this makes microvolume spectrophotometers, which allow the measurement of tiny drops of sample (typically 1-2 µL), the instrument of choice for quantification of DNA, RNA and proteins using absorbance.

There are many microvolume spectrophotometers available on the market. In this technical note, we compare the performance of the Colibri+ LB 916 with three other instruments, the NanoDrop™ One from Thermo Fisher Scientific and the DS-11 from DeNovix, and with the Nabi from MicroDigital.

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Colibri+ Microvolume spectrophotometer

The Colibri+ is a high-performance microvolume spectrophotometer for the determination of the concentration of DNA, RNA, and proteins in microvolumes (typically 1 µL) using absorbance.

The system has been designed to help you accelerate your research:

- Ultra-fast measurement (<3 s/sample).
- Intuitive operation.
- High reliability (CV < 1%).
- Broad detection range (2-20,000 ng/µL ds DNA).
- Broad wavelength range (190-1000 nm).
- Easy pipetting of samples thanks to its Sample Guide Light.

Whether you are performing real-time PCR, sequencing/NGS or cloning experiments, the innovative features of the Colibri+ will help you to advance your application.

Materials

- Colibri+ from Berthold Technologies (Id. Nr. 73179).
- NanoDrop™ One from Thermo Fisher Scientific (Cat # ND-ONE-W).
- DS-11 from Denovix.
- Nabi from MicroDigital.
- Salmon Sperm DNA, sheared (10 mg/mL) from Invitrogen™ (Cat # AM9680).
- Nuclease-free water to prepare the dilution series of DNA.
- Pipettes and pipette tips of various sizes.

Methods

A dilution series of dsDNA was prepared by serially diluting ½ the stock solution (10 mg/mL). This resulted in the following concentrations: 10000, 5000, 2500, 1250, 625, 312.5, 156.25, 78.13, 39.06, 19.53, 9.77, 4.88, and 2.44 ng/µL.

Each DNA concentration was measured in triplicate with all instruments. In order to reduce the contribution of pipetting error, the volume measured was 2 µL in all cases.

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The following parameters were calculated:

- To assess the accuracy of the instruments, the absolute value of the difference between the measured dsDNA concentration and the theoretical concentration was calculated and expressed as percent of the theoretical concentration. This parameter is displayed as Error (%) in the tables.
- To assess the reproducibility of the instruments, the coefficient of variation of the measured dsDNA concentration was calculated (standard deviation expressed as percent of the theoretical concentration). This parameter is displayed as CV (%) in the tables.

Instrument settings

The measurement was performed with default settings for dsDNA in all instruments, with baseline correction active. In the Colibri+, a pathlength of 0.5 mm was used for concentrations of 625 ng/µL and lower, and 0.05 mm for concentrations of 1250 ng/µL and higher.

Results

Reproducibility results are summarized in Table 1. The reproducibility of the Colibri+ is comparable to that of the NanoDrop™ One and DS-11 in the whole concentration range, with excellent CV% values at concentrations above 20 ng/µL and is better than that of the Nabi. In all instruments, CV% increases notably at the lowest concentrations.

Accuracy results are summarized in Table 2. As shown by the calculated errors, the accuracy of the Colibri+ is better than that of the other instruments tested at concentrations of 312.5 ng/µL and below (with the exception of 4.88 ng/µL). Error is considerably high in all instruments at low concentrations, and it increases as the concentrations approach the lowest concentrations. In fact, some instruments (DS-11 and Nabi) already exhibit errors above 10% at concentrations of 39.06 ng/µL, while in the Colibri+, this happens only at the lowest concentrations (9.77 ng/µL and below). At higher concentrations the error is comparable in all instruments, and typically under 5%.

ng/µL dsDNA	CV% Colibri+	CV% ND One	CV% DS-11	CV% Nabi
10000	0.4	1.7	0.2	8.7
5000	0.3	0.8	0.3	6.9
2500	0.5	0.2	0.4	0.7
1250	0.4	1.5	0.3	1.0
625	0.1	0.3	0.1	0.8
312.50	0.1	0.1	0.1	0.9
156.25	0.6	1.2	0.5	0.3
78.13	1.2	0.3	1.0	0.9
39.06	0.6	0.1	1.7	1.6
19.53	2.1	1.0	2.2	0.6
9.77	5.8	1.9	5.9	3.3
4.88	8.6	5.7	7.1	8.0
2.44	14.2	15.2	13.4	21.6

***Table 1**: Reproducibility of the measurement of dsDNA concentrations. All measurements in triplicate.*

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ng/µL dsDNA	Colibri+		NanoDrop™ One		DS-11		Nabi	
Theoretical	Measured	Error (%)	Measured	Error (%)	Measured	Error (%)	Measured	Error (%)
10000	9557.4	4.4	9489.9	5.1	10300.7	3.0	10462.3	4.6
5000	4650.9	7.0	5248.6	5.0	5047.5	0.9	5572.3	11.4
2500	2435.6	2.6	2504.6	0.2	2485.7	0.6	2618.8	4.8
1250	1254.7	2.5	1276.1	2.1	1305.7	4.5	1370.0	9.6
625	657.1	5.1	639.5	2.3	651.3	4.2	655.5	4.9
312.50	316.0	1.2	293.9	6.0	327.3	4.9	332.0	6.3
156.25	163.6	4.9	146.6	6.2	169.5	8.6	169.8	8.7
78.13	81.4	4.4	72.0	7.8	84.5	8.3	84.7	8.4
39.06	42.1	7.9	35.5	9.2	44.4	14.0	44.8	14.6
19.53	21.9	9.3	16.0	18.3	23.0	14.9	24.9	27.7
9.77	11.3	12.6	8.1	17.0	11.6	16.0	14.1	44.7
4.88	6.3	25.8	4.1	16.5	5.9	17.8	8.0	64.5
2.44	3.3	30.9	1.5	40.2	3.9	55.4	4.6	89.8

***Table 2**: Accuracy in the measurement of dsDNA concentrations. Error was calculated as the absolute value of the difference between the measured concentration and the theoretical concentration, expressed as percent of the theoretical concentration. All measurements in triplicate.*

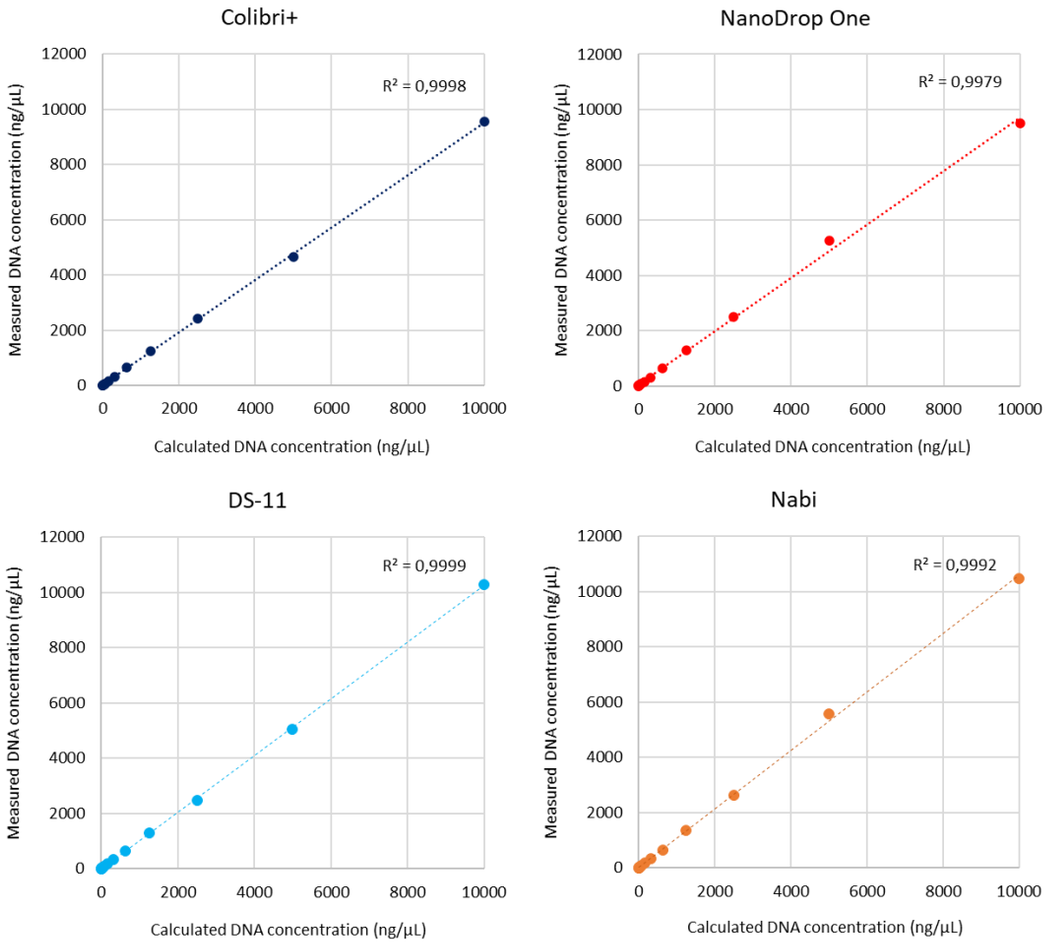

***Figure 1**. Linearity in the whole concentration range. All measurements in triplicate.*

Figure 1 shows the linearity of all instruments in the whole concentration range tested (2.5-10,000 ng/µL dsDNA). All instruments displayed excellent linearity, with the DS-11 and the Colibri+ obtaining the best correlation scores (R^2 = 0.9999 and 0.9998 respectively).

Conclusions

The Colibri+ has excellent accuracy, reproducibility, and linearity. Generally speaking, it has a performance comparable to that of the NanoDrop™ One and the DS-11 and has better accuracy in some concentration ranges. It has also better performance than the Nabi.

The high performance of the Colibri+, combined with fast measurement speeds (<3 s per sample), broad wavelength and concentration range, and easy and reliable pipetting, make the Colibri+ an excellent microvolume spectrophotometer for most laboratories working with nucleic acids.

References

1. Huss, V.A.R., Festl, H., Schleifer, K.H. Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Systematic and Applied Microbiology, 1983; 4(2): 184-192.

Not for use in diagnostic procedures.

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Application Note

MEDIUM-THROUGHPUT MICROVOLUME DNA QUANTIFICATION ON BERTHOLD TRISTAR MULTIMODE READERS

USING THE µDROP™ PLATE TO INCREASE LAB PRODUCTIVITY OF NUCLEIC ACID QUANTIFICATION

Abstract

Absorbance measurements of DNA, RNA and proteins are often performed using a microvolume spectrophotometer. However, this method can normally only be used to measure single samples, limiting the productivity of laboratories that need to analyse large numbers of samples. While microplate readers offer much higher throughput, standard microplates require large sample volumes that are often not available. The µDrop™ plate offers a solution to this problem. With 16 sample wells and a sample volume of just 2 µL, throughput is significantly increased compared to a microvolume spectrophotometer. Combined with a plate reader such as the Tristar multimode readers, it provides a medium throughput solution for nucleic acid quantification in combination with small sample volumes.

Introduction

DNA quantification is an important pre-analytical method, which is of great importance for many molecular biology analytical methods and can even determine their success. It is also a routine technique in procedures for translational research such as Next-Generation Sequencing (NGS), Polymerase Chain Reaction (PCR) or Real-Time PCR (quantitative PCR; qPCR), cloning or transfection, which initiates the subsequent workflow.

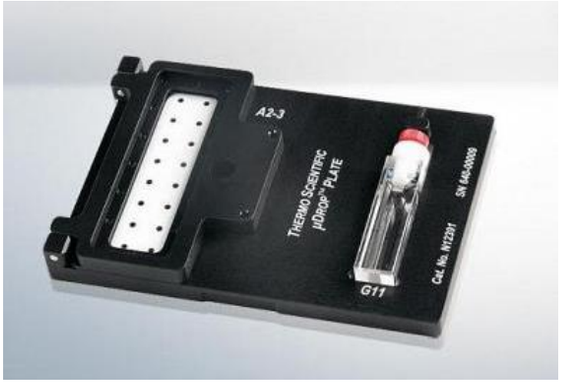

Figure 1: The µDrop™ plate can be used for DNA quantification using absorbance at 260 nm in a microplate reader using only 2 µL of sample. It also features a cuvette port for increased flexibility.

The most popular DNA quantification methods are based on UV-Vis- or fluorescence spectroscopy. Absorbance at 260 nm has been the method of choice for routine quantification of DNA and RNA since decades. It is simple and convenient to use as no

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further sample treatment (other than DNA extraction) is required. However, it is not very specific (it measures all nucleic acids as a whole) and it is sensitive to contaminants, so it demands very pure DNA to be accurate. Many of those contaminants can be estimated by measuring the absorbance of the sample at wavelengths other than 260 nm (usually at 230, 280 and 340 nm).

Absorbance of DNA samples at 260 nm is currently measured most often using a microvolume spectrophotometer (such as the Colibri+ from Berthold), but it is also possible to use a microplate reader. Microplate readers can measure many samples in a short time (typical plate formats are 96- and 384-well), but they require larger sample volumes for the measurement than a microvolume spectrophotometer (up to 50 µL in standard 96-well plates, less in other microplate formats). However, there is also the µDrop™ plate. It uses small sample volumes (from 2 µL). While it doesn't have as many sample positions as standard microplates (16 instead of 96), it offers a good compromise between sample volume and throughput. It also features a cuvette port, which allows to measure cuvettes in the microplate reader.

In this Application Note we demonstrate the suitability of the Tristar Multimode Microplate Readers or the quantification of dsDNA using the µDrop™ plate.

Materials

- Tristar 5 Multimode Microplate Reader, Berthold Technologies (Id. Nr. 69185-15).
- Tristar 3 Multimode Microplate Reader, Berthold Technologies (Id. Nr. 69173-30).
- µDrop™ microvolume plate (Id. Nr. 64154).
- Invitrogen™ Salmon Sperm DNA, sheared (10 mg/mL), Thermo Fisher (Cat. #AM9680).
- Nuclease-free water.
- Tubes of various volumes.
- Pipettes and pipette tips (various volumes).

Methods

The DNA stock was diluted to a concentration of 1600 ng/µL using TE buffer. A ½ dilution series was prepared using this diluted stock, producing the following DNA concentrations: 1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ng/µL DNA.

To perform the blank measurement, 2 µL TE buffer were pipetted in duplicate in each spot of the µDrop™ plate and absorbance was measured at 260 and 340 nm. After removing the TE buffer from the plate, 2 µL of each DNA concentration were pipetted in the µDrop plate and absorbance was measured at the same wavelengths.

The plate was measured with the settings detailed under "Instrument settings". The absorbance at 340 nm was used for baseline correction, and blank values were subtracted from the absorbance values of the DNA solutions at the corresponding position. Data were exported to xls format, and standard curves were drawn in Excel.

In addition, the spectrum of the 1600 ng/µL standard was measured using a High-resolution scan (see "Instrument settings").

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Developed for high flexibility and equipped with the proprietary ONE-4-ALL optical system, the Tristar Serie combines the user friendliness of a multimodal optical system with the sensitivity and performance of a dedicated optical device. You can choose between the affordable Tristar 3 and the more advanced and flexible Tristar 5. The Tristar series provides you with flexibility for today, tomorrow, and beyond.

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- High-sensitivity Luminescence
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- Time-Resolved Fluorescence (TRF)
- Time-Resolved FRET (TR-FRET / HTRF®)
- Fluorescence Polarization
- AlphaScreen®
- Top and Bottom Reading
- Incubation

Instrument settings

Tristar 3:

- Reading mode: Absorbance
- Counting time: 0.10 s
- Use: Filters
- Aperture: 3 – Rd2
- Measurement Filters: 240/10, 260/10, 280/5, 340/26.

Tristar 5 (DNA concentration):

- Reading mode: Absorbance
- Counting time: 0.10 s
- Use: Monochromator
- Beam size: Narrow
- Aperture: Default
- Meas. Wavelength: 230, 260 280 and 340 nm
- Meas. Slit Width: 5 nm

Tristar 5 (spectrum):

- Reading mode: Absorbance Spectral scan
- Counting time: 0.10 s
- Scanning quality: High resolution (slow)
- Beam size: Narrow
- Aperture: 3 - Rd 2
- Meas. Start Wavelength: 230 nm
- Meas. End Wavelength: 340 nm
- Increment Wavelength: 1 nm
- Meas. Slit Width: 5 nm

For the best performance we recommend creating a new plate profile for the µDrop™ plate using the Plate Editor and the following parameters:

- Number of rows: 8
- Number of columns: 12
- Height of plate: 14.40 mm
- Stacking height: 13.50 mm
- Distance from corner and from well to well: 15.10 mm (top), 11.60 (left), 9.00 (right), 9.0 (bottom)

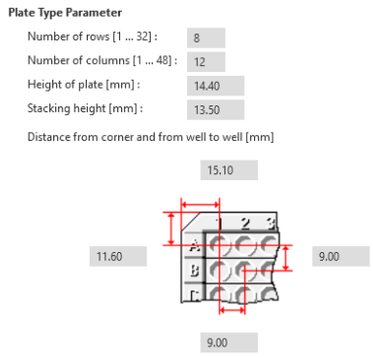

Figure 2: parameters for the µDrop™ plate as seen in the Plate Editor.

Results

The absorbance spectrum measured with the Tristar 5 was clean and with the expected shape for a high-purity DNA (figure 3).

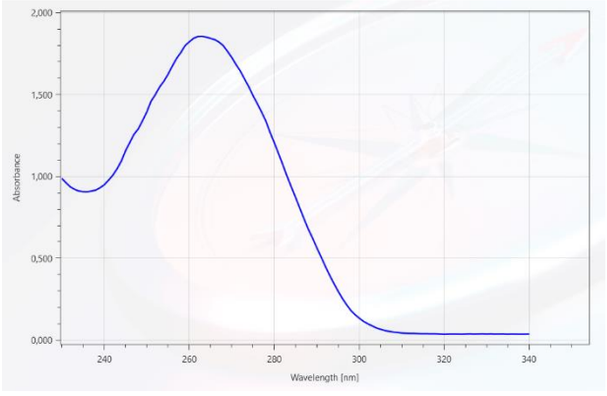

Figure 3: absorbance spectrum of the 1600 ng/µL DNA solution, measured at high resolution (1 nm scan).

The DNA standard curves obtained are displayed in figure 4. Linearity is excellent with both the Tristar 5 and the Tristar 3, with R^2 values of 0.9996 and 0.9993, respectively.

In preliminary measurements, the concentrations in positions pipetted first were overestimated (data not shown), probably due to evaporation during the time spent pipetting subsequent sample positions, as sample volume is small (2 µL) and the samples are exposed to air until the last sample is pipetted and the plate is closed. Hence, we recommend using a multichannel pipette to pipet blanks and samples in the µDrop™ plate.

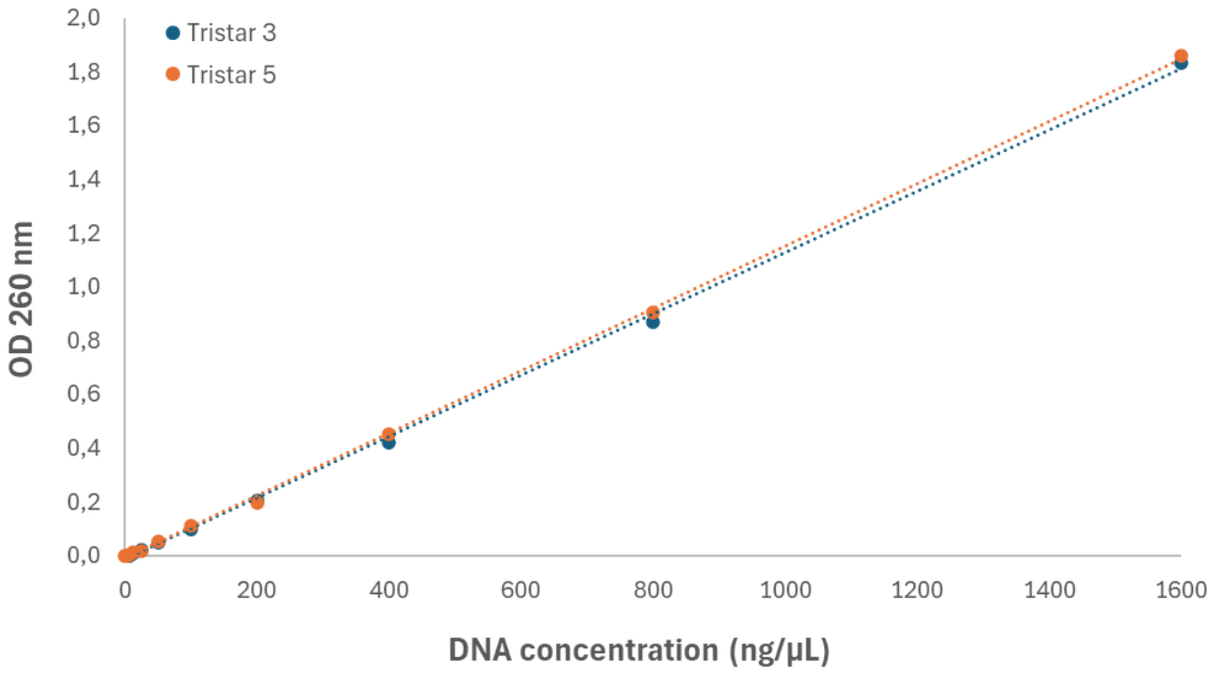

Figure 4: Dilution series of the 1600 ng/µL DNA solution, down to 1.563 ng/µL. Each standard was measured in duplicate in the µDrop™ plate.

Discussion and conclusions

Quantification of nucleic acids is very often performed using a microvolume spectrophotometer. However, this method suffers from low throughput, as samples have to be measured one by one. The µDrop™ plate, with 16 sample positions and a minimum sample volume of 2 µL, offers a higher throughput and, as displayed in the graphs above, can be used in both the Tristar 5 and Tristar 3 microplate readers. While it is possible to pipet the drops using a single-channel pipette, using a multi-channel pipette increases throughput and, most importantly, prevents inaccuracies due to evaporation.

All in all, the combination of the µDrop™ plate with the Tristar microplate readers is a useful tool for the quantification of nucleic acids for a range of applications, such as PCR, cloning, sequencing and others. It offers the means to simplify the workflow, while saving sample and improving the accuracy of the measurement.

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Application Note

QUANTIFYING DNA WITH THE QUANT-IT™ PICOGREEN® dsDNA KIT AND BERTHOLD TRISTAR MULTIMODE READERS

FAST, SIMPLE, AND ACCURATE NUCLEIC ACID QUANTIFICATION

Abstract

DNA quantification is mostly done by absorbance at 260 nm, but suffers from low throughput, sensitivity, and specificity. The Quant-iT™ PicoGreen® dsDNA reagent is specific for dsDNA and is suitable for microplate readers. In combination with the Tristar 3 and Tristar 5 Multimode Microplate Readers, it allows the specific quantification of dsDNA in 96-well plates achieving a limit of detection below 0.1 pg/µL.

Introduction

DNA quantification is an important pre-analytical method, which is of great importance for many molecular biological analysis methods and can even determine their success. It is also a routine technique in procedures for translational research such as Next-Generation Sequencing (NGS), Polymerase Chain Reaction (PCR) or Real-Time PCR (quantitative PCR; qPCR), cloning or transfection, which initiates the subsequent workflow.

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The most popular DNA quantification methods are based on UV-Vis- or fluorescence spectroscopy. Both methods have advantages and disadvantages.

Quantification using absorbance

Absorbance at 260 nm has been the method of choice for routine quantification of DNA and RNA since decades. It is simple and convenient to use as no further sample treatment (other than DNA extraction) is required. However, it is not very specific (it measures all nucleic acids as a whole) and it is sensitive to contaminants, so it demands very pure DNA to be accurate. Many of those contaminants can be estimated by measuring the absorbance of the sample at wavelengths other than 260 nm (usually at 230, 280 and 340 nm).

Absorbance of DNA samples at 260 nm is currently most often measured using a microvolume spectrophotometer, but it is also possible to use a microplate reader. Microplate readers can measure many samples in a short time (typical plate formats are 96- and 384-well), but they require larger sample volumes for the measurement than a microvolume spectrophotometer (up to 50 µL in standard 96-well plates, less in other microplate formats). However, microvolume microplates are available which use small sample volumes (usually 2 µL). While they don't

have as many sample positions as standard microplates (normally 16 instead of 96), they offer a good compromise between sample volume and throughput.

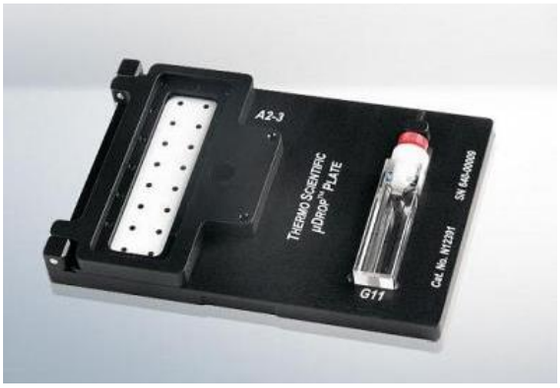

Figure 1: The µDrop plate can be used for DNA quantification using absorbance in a microplate reader using only 2 µL of sample and also features a cuvette port for increased flexibility.

Quantification using fluorescence

The use of fluorescent dyes permits the quantification of DNA with much higher sensitivity than measuring absorbance of DNA itself (typically 10-1000 times higher, depending on the specific methods compared). In addition, specific dyes can be used to stain only specific types of nucleic acid, such as dsDNA or RNA, thereby increasing the specificity of the quantification and reducing the effect of contaminants. However, fluorescence-based methods are more expensive than measuring absorbance at 260 nm, often require a standard curve to be prepared, and do not provide a direct estimate of the presence of contaminants (which may be important, for example, to evaluate the possible effects on downstream methods). Fluorescence measurement is performed using a microplate reader or a single tube fluorometer.

Quant-iT™ PicoGreen® dsDNA reagent is an ultrasensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. Detecting and quantitating small amounts of DNA is extremely important in a wide variety of biological applications. The PicoGreen dsDNA quantitation reagent and kits are ideal for PCR-based assays, microarray samples, DNA damage assays, enzyme activity assays, genomic DNA quantitation, measuring dsDNA in complex mixtures, and viral DNA quantitation.

In this Application Note we report the suitability of the Tristar Multimode Microplate Readers to quantify dsDNA using the Quant-iT™ PicoGreen® dsDNA reagent and the recommended settings for this method.

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Developed for high flexibility and equipped with the proprietary ONE-4-ALL optical system, the Tristar Serie combines the user friendliness of a multimodal optical system with the sensitivity and performance of a dedicated optical device. You can choose between the affordable Tristar 3 and the more advanced and flexible Tristar 5. The Tristar series provides you with flexibility for today, tomorrow, and beyond.

- Monochromator Technology
- High-sensitivity Luminescence
- BRET
- UV/VIS Absorbance
- UV/VIS Fluorescence
- UV/VIS FRET
- Time-Resolved Fluorescence (TRF)
- Time-Resolved FRET (TR-FRET / HTRF®)
- Fluorescence Polarization
- AlphaScreen®
- Top and Bottom Reading
- Incubation

Materials

- Tristar 3 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69173-30).
- Tristar 5 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69185-15).
- Quant-iT™ PicoGreen® dsDNA Assay Kit from Invitrogen™ (Cat. # P7589).
- Black 96-well microplates from Berthold Technologies (Id. Nr. 23302).
- Tubes of various volumes.
- Pipettes and pipette tips (various volumes).

Instrument settings

- Reading mode: Fluorescence Endpoint
- Excitation filter: 485/14
- Emission filter: 535/25
- Counting time: 0.1 s
- All other settings with default values

Methods

Reagents were prepared following the manufacturer's instructions. Using the DNA standard included with the kit, two different standard curves were prepared: a high range standard curve, which could be used to quantify high concentration samples, and a low range standard curve, which could be used to quantify low concentration samples.

Equal volumes of standard and Quant-iT™ PicoGreen® reagent were mixed for each standard point. The final concentrations of each curve were the following ones:

- High range: 1, 10, 100, 250, 500, 750 and 1000 pg/µL dsDNA.
- Low range: 0.025, 0.25, 2.5, 5, 10, 20 and 25 pg/µL dsDNA

The mix was incubated for 5 minutes at room temperature in the dark. 200 µL of each mix were pipetted in triplicate in the wells of a black 96-well TE buffer was used as blank.

The plate was inserted in the multimode reader and measured with the settings detailed above. Blank values were subtracted from the values of the standard. Data were exported from the ICE software to xls format, and standard curves were drawn in Excel.

Results

The High Range and Low range standard curves of the Tristar 3 and Tristar 5 Multimode Microplate Readers are displayed in Figure 2. All curves exhibit excellent linearity.

The calculated Limit of Detection in 96-well microplates was 0.069 pg/µL dsDNA (13.8 pg/well) for the Tristar 3 and 0.083 pg/µL dsDNA (16.6 pg/well) for the Tristar 5. When the measurement was performed in 384-well plates, the calculated Limit of Detection was 0.272 pg/µL dsDNA (19.0 pg/well) for the Tristar 3 and 0.110 pg/µL dsDNA (7.6 pg/well) for the Tristar 5.

With the settings used, a full 96-well plate can be measured in 33 seconds.

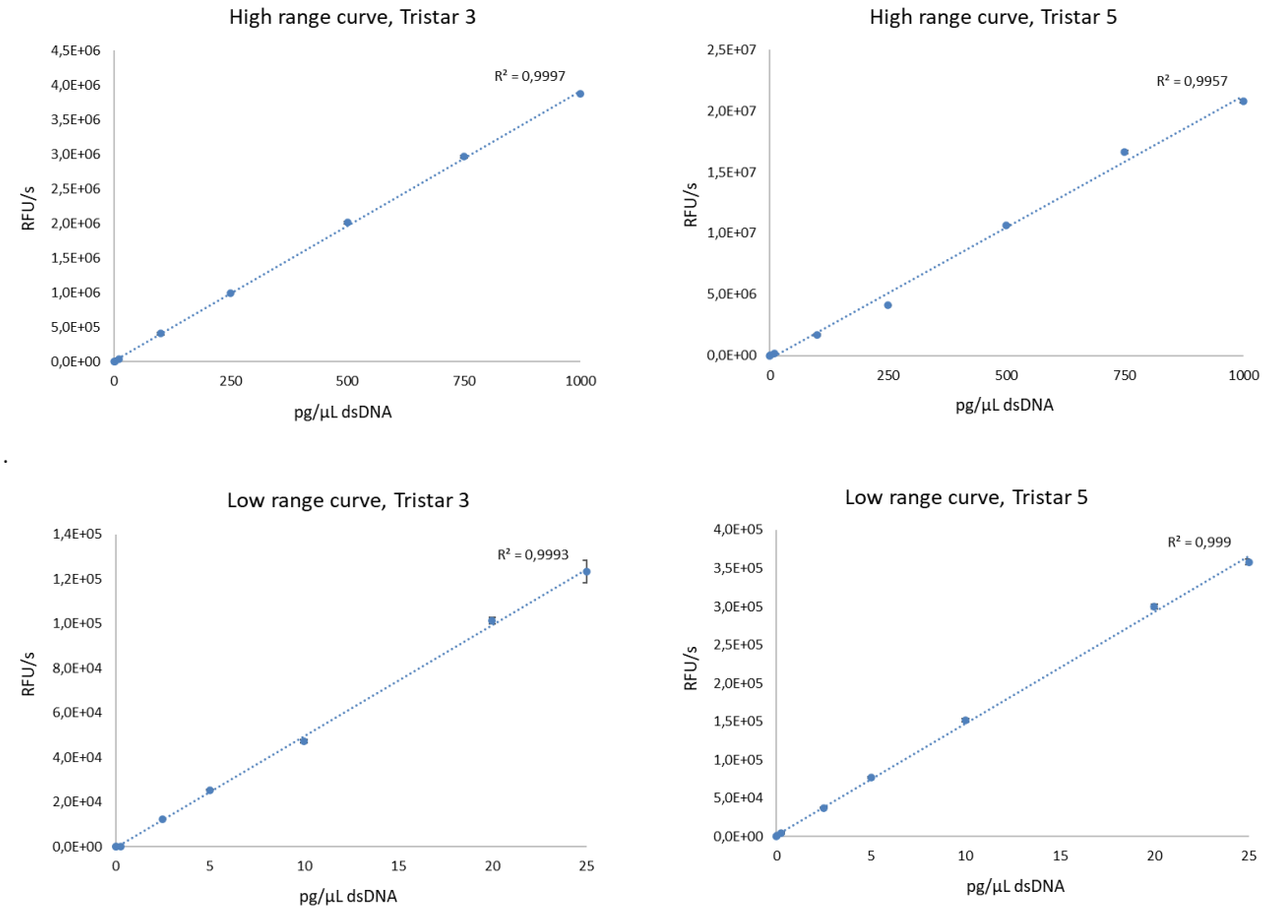

Figure 2: dsDNA standard curves measured with a Tristar 3 (left) and Tristar 5 (right) Multimode Reader with standard fluorescein filters. All measurements in triplicate. Data are average ± SEM.

Discussion and conclusions

Quantification of dsDNA is very often performed using a microvolume spectrophotometer. However, this method suffers from low throughput, as samples have to be measured one by one, and of low sensitivity, as the limit of detection is typically 2 ng/µL dsDNA (2000 pg/µL). While using the Quant-iT™ PicoGreen® dsDNA reagent involves some increase in costs and preparation time (as a standard curve must be prepared and measured), when used to measure dsDNA concentrations in the Tristar Multimode Microplate Readers, it allows to measure 96 wells under a minute while achieving a limit of detection below 0.100 pg/µL. This represents an improvement of sensitivity of 20,000-fold over the detection limit of a microvolume spectrophotometer. The limit of detection obtained with Tristar instruments is clearly better than the typical result for microplate readers which, according to the kit insert, is of 0.25 pg/µL.

The results demonstrate the high performance of the Tristar Multimode Microplate Readers for the quantification of dsDNA using the Quant-iT™ PicoGreen® dsDNA reagent. The system not only enables dsDNA concentration measurements over a wide dynamic range but can also help increase the throughput of this qualification step in important applications such as PCR assays, microarray samples, DNA damage assays, enzyme activity tests, or the quantification of viral DNA.

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Application Note

QUANTIFICATION OF DNA FOR NGS WITH THE QUBIT™ ASSAY AND TRISTAR MULTIMODE READERS

Increase your throughput and facilitate repeat measurements

Abstract

Next generation sequencing (NGS) workflows require highly accurate DNA quantification. The widely used Qubit™ assay is normally analysed with a Qubit™ fluorometer. However, they are performed in tube format. This makes the application suitable only for laboratories processing a small number of samples. In this application note the Qubit™ dsDNA BR kit is adapted to microplate format using the Tristar multimode readers. With only 4 standard points, the tested settings provide high throughput and high performance.

Introduction

Next generation sequencing (NGS) is a nucleic acid sequencing technology that enables the sequencing of thousands of genes simultaneously in multiple samples. This provides valuable information for a broad range of fields and applications, from cancer research to prenatal testing. There are many companies offering NGS solutions, but the market is currently dominated by Illumina® and Thermofisher Scientific [1].

NGS is a very powerful technology, but it has also strict requirements concerning sample quality and handling. Accurate quantification and proper quality check of next-generation sequencing libraries is key to a successful sequencing run and determines data quality and overall data yield: insufficient input will lead to under-clustered flow cells, and excess input will lead to over-clustered flow cells, compromising the quality of results. In many NGS workflows, DNA quantification is performed twice: before and after library preparation.

While DNA quantification using UV spectrophotometry is very popular for other applications, it is not recommended for NGS. Depending on the library preparation kit used, different DNA quantification methods are favoured, and fluorometric quantification using assays such as Qubit™ and Picogreen® are recommended in many cases [2]. We have already covered the use of Quant-iT™ Picogreen® with Tristar multimode readers in a previous application note [3]. In the present Application Note we are going to focus on the Qubit™ assay.

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The Qubit™ assay is intended to be performed in Qubit™ fluorometers. However, these fluorometers use tubes as sample format, and this limits throughput. Although Quant-iT™ assays are most suitable to be measured in microplates, many scientists and technicians have used Qubit™ for years and are happy to keep using it, even if sample throughput increases and requires moving to microplate format. Fortunately, the Qubit™ assay can be easily adapted to the microplate format.

In this Application Note we report that the Qubit™ dsDNA BR assay kit is easily adapted to 96-well microplate format using the Tristar Multimode Microplate Readers.

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- Fluorescence Polarization
- AlphaScreen®
- Top and Bottom Reading
- Incubation

Materials

- Tristar 3 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69173-10).
- Tristar 5 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69185-15).
- Qubit™ dsDNA BR Assay Kit from ThermoFisher (Cat. # Q32850).
- Invitrogen™ Salmon Sperm DNA, sheared (10 mg/mL) from ThermoFisher (Cat. #AM9680).
- Black 96-well microplates from Berthold Technologies (Id. Nr. 23302).
- LightCompass® software from Berthold Technologies.
- Tubes (various volumes).
- Pipettes and pipette tips (various volumes).

Instrument settings

- Reading mode: Fluorescence Endpoint
- Excitation filter: 485/14
- Emission filter: 535/25
- Counting time: 0.1 s
- All other settings with default values

Methods

Reagents were prepared following the manufacturer's instructions. In order to further extend the concentration range of the assay, a more concentrated DNA standard was used instead of the one included with the kit. The DNA stock was diluted to a concentration of 160 ng/µL using TE buffer. To prepare the standard curve, a ½ dilution series was prepared using this diluted stock, producing the following DNA concentrations: 160, 80, 40, 20, 10, 5, 2.5, and 1.25 ng/µL DNA. TE buffer was used as blank.

In addition, to assess the performance of the assay, 3 "unknown" samples were prepared with concentrations different to that of the standard points: 120, 30 and 7.5 ng/µL DNA.

The recommended reaction volume of the Qubit™ assay is 200 µL, which fits perfectly with the working volume of most standard 96-well microplates. According to the instructions of the kit, 10 µL of each standard (1-20 for samples) have to be mixed were mixed with 190 µL of working solution (180-199 for samples). In this case, we used 10+190 for both standards and samples. To perform the assay in triplicate, triple volumes (plus 10 extra microliters, to ensure enough volume was available) were mixed in 1.5 mL microcentrifuge tubes and vortexed vigorously for 5 seconds. After an incubation of 5 minutes at room temperature protected from light, 200 µL of each standard and sample were pipetted in triplicate in the wells of the 96-well microplate. Then the microplate was measured immediately in the microplate reader using the settings described above.

One critical point of the assay is the curve fitting algorithm. According to the available documentation, Qubit™ fluorometers use a "modified Hill plot" algorithm, but no details are given about the modifications. To find out the best curve fitting algorithm, accuracy of the following fitting algorithms was compared: 4 parameter, linear, point-to-point and polynomial (2nd and 3rd degrees).

Results

Fitting algorithm

In the User Guide of the kit, it can clearly be seen that fitting algorithm used by Qubit™ fluorometers is not linear, but it produces a slightly curved line [4]. Considering this, it's not surprising that using linear regression plots a line that is relatively far from some of the standard points (Fig. 1); on the other hand, curve fitting algorithms such as 4 parameter logistic show a much better fit (Fig. 2). When expressing measurements as relative fluorescence, both the Tristar 3 and the Tristar 5 produce very similar curves (Fig. 1 and 2).

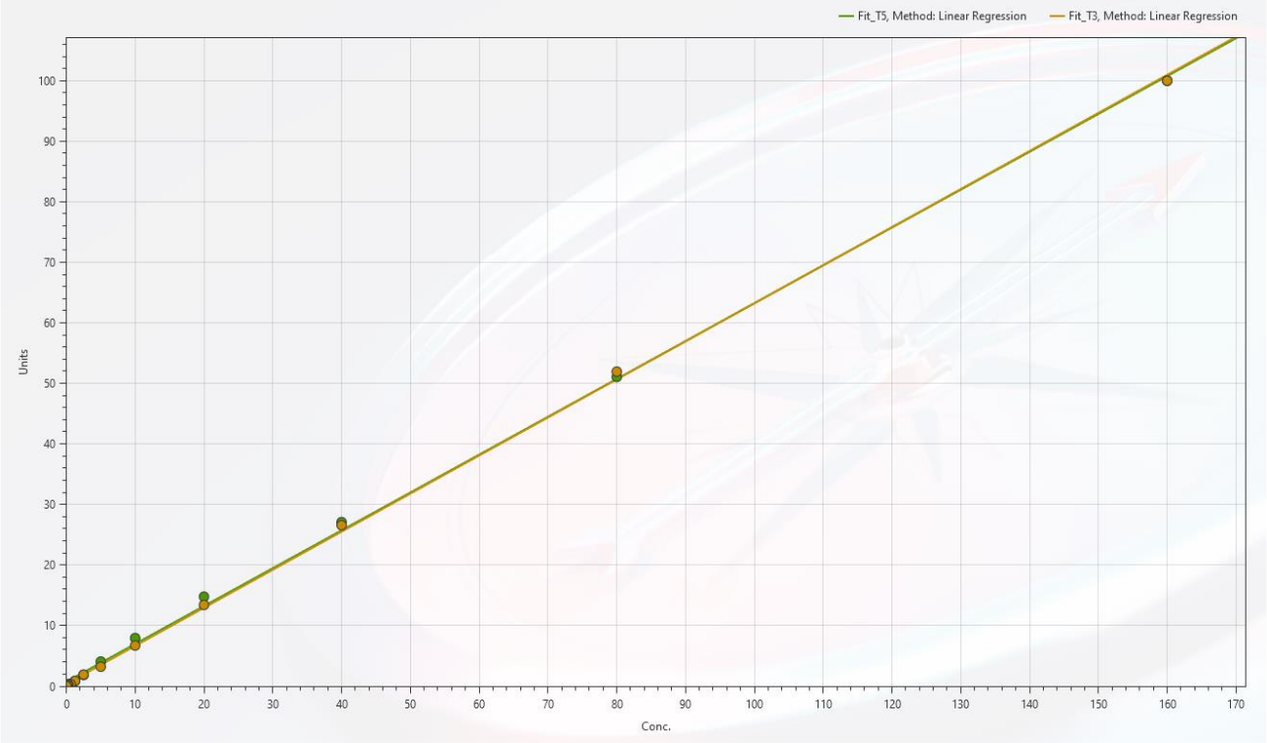

Figure 1. Standard curves generated by linear regression in the Tristar 5 and Tristar 3 instruments. Measurement values expressed as % of the maximum fluorescence, concentrations in ng/µL. All data points measured in triplicate.

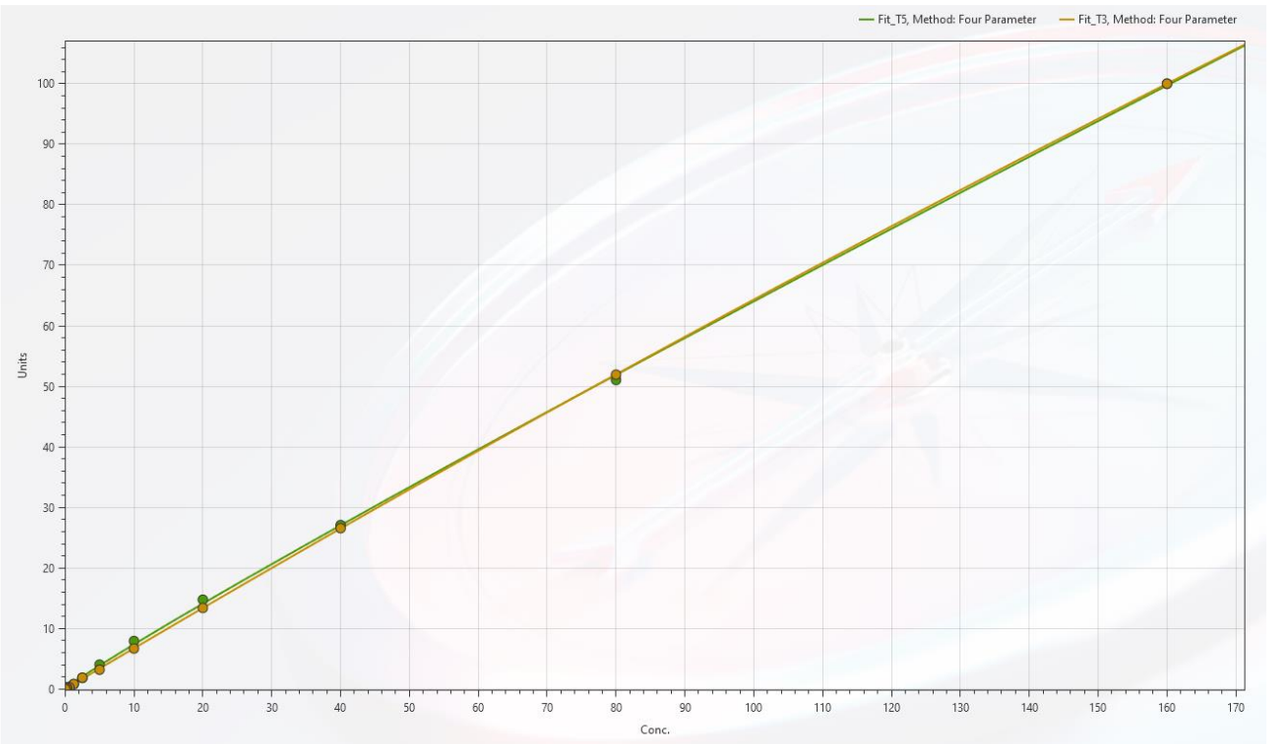

Figure 2. Standard curves generated by 4 parameter logistic regression in the Tristar 5 and Tristar 3 instruments. Measurement values expressed as % of the maximum fluorescence, concentrations in ng/µL. All data points measured in triplicate.

In the concentration ranges tested, all fitting algorithms provided good results (Table 1), with an average error <5% in all cases. Overall, the best results were obtained with 4 parameter logistic, which produced results with an average error of 2.3% and no error going above 2.8% at any data point. Considering this error comes from 3 different pipetting steps plus measurement error, this level of accuracy is remarkably good.

Tristar 5					
Theoretical concentration	4 Parameter Logistic	Linear Regression	Point-to-point	Polynomial (2nd degree)	Polynomial (3rd degree)
120	116.67	117.26	117.54	116.01	120.23
30	29.18	31.02	28.63	29.83	28.33
7.5	7.57	8.05	7.18	8.03	7.56

Tristar 3					
Theoretical concentration	4 Parameter Logistic	Linear Regression	Point to point	Polynomial (2nd degree)	Polynomial (3rd degree)
120	117.84	118.97	118.65	117.78	117.93
30	29.17	30.37	29.17	29.19	29.14
7.5	7.36	7.36	7.52	7.31	7.37

Table 1. Calculated dsDNA concentrations using different curve fitting methods. All concentrations in ng/µL. All data points measured in triplicate.

Number of standard points

Measuring the Qubit™ assay in a Qubit™ fluorometer requires only 2 standard points: 100 and 0 ng/µL, but more data points are required for accurate fitting of the curve in most methods tested above. However, using a 9-point standard curve involves considerable handling time and reagent consumption. In order to optimize the protocol, curves with 5 and 4 standard points were used and compared to results obtained with the 9-point standard curve. The standard points chosen for each reduced standard curve were:

5 points: 160, 40, 10, 2.5, 0 ng/µL
4 points "high": 160, 40, 5, 0 ng/µL
4 points "low": 160, 40, 1.25, 0 ng/µL

Tristar 5				
Theoretical concentration	**9 points**	**5 points**	**4 points "high"**	**4 points "low"**
120	116.67	116.11	116.27	114.57
30	29.18	28.86	29.06	29.58
7.5	7.57	7.53	7.59	8.49

Tristar 3				
Theoretical concentration	**9 points**	**5 points**	**4 points "high"**	**4 points "low"**
120	117.84	117.78	117.03	117.76
30	29.17	29.10	29.28	29.09
7.5	7.36	7.29	7.67	7.34

Table 2. Calculated dsDNA concentrations using standard curves with different number of data points (see above). All concentrations in ng/µL. All data points measured in triplicate.

Reducing the number of concentrations for the standard curve down to 4 produces no significant loss of accuracy, with calculated concentrations very similar to the ones produced using 9 points. Between the 2 combinations of 4 points tested, the "high" one, using 5 instead of 1.25 ng/µL to draw the curve, provides the best results in the Tristar 5. In Tristar 3, both curves produce similar results.

Discussion and conclusions

Next Generation Sequencing (NGS) is a demanding method that requires very accurate quantification of dsDNA at several steps of the workflow. In many cases, fluorescent methods such as Quant-IT™ and Qubit™ are recommended for the quantification. Qubit™ has been designed to be measured in a Qubit™ fluorometer, but tube format limits throughput. Many laboratories would prefer adapting the Qubit™ assay to microplate format instead of migrating to different methods such as Quant-IT™.

In this application note we demonstrate that the Qubit™ dsDNA BR assay kit can be easily adapted to 96-well format using Tristar multimode readers and LightCompass® software The two members of the Tristar family, Tristar 5 and Tristar 3, deliver very similar results. Using a 4-parameter logistic algorithm, 4 standard points are sufficient to provide good performance in the concentration range tested: This allows rapid quantification of 44 samples in duplicate (28 samples in triplicate) in a single 96-well plate. For laboratories working with samples of lower concentration, the Qubit™ dsDNA HS assay kit is available. Our expectation is that it will also work very well in microplate format with the Tristar multimode readers.

Taking everything into account, the Tristar multimode readers are a perfect solution for measuring the Qubit™ dsDNA BR assay in microplate format. Using the curve fitting algorithms and standard points detailed in this application note, they offer a DNA quantification solution with high reliability and throughput. This makes adapting the assay protocol to the microplate format a valuable tool for laboratories that use Qubit™ assays for DNA quantification and do not want to switch to another method.. For laboratories considering other methods, the Quant-IT™ Picogreen® dsDNA assay kit also provides excellent performance in the Tristar multimode readers [3].

References

1. DNA sequencing: how to choose the right technology (2021). Front Line Genomics, accessed 26.9.2024. https://frontlinegenomics.com/dna-sequencing-how-to-choose-the-right-technology/
2. Library quantification and quality control quick reference guide (2023). Illumina, accessed 23.9.2024. https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000001247
3. Quantifying DNA with the Quant-IT™ Picogreen® dsDNA kit and Berthold Tristar multimode readers (2023). Berthold Technologies. Available for download at

https://www.berthold.com/fileadmin/DownloadsUnprotected/application-notes/AN942_010-Quant-iT_Picogreen_dsDNA_Tristar_Series.pdf

4. User Guide of the Qubit™ dsDNA BR Assay Kit (2022). Pub. No. MAN0002325, Rev. B0. Thermo Fisher Scientific.

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Application Note

MINIATURISATION OF THE QUBIT™ ASSAY WITH TRISTAR MULTIMODE READERS

SMALLER SAMPLE FORMATS REDUCE COSTS AND INCREASE THROUGHPUT

Abstract

Fluorescence-based DNA quantification methods offer higher sensitivity and specificity than UV spectrophotometry. Qubit™ assays are a widely used method of this type. They are performed in tube format, being suitable only for laboratories processing low numbers of samples We have already demonstrated that they can be easily adapted to the 96-well format. Moving to even smaller formats not only enables a further increase in throughput, but also cost savings. In this application note we show that the Qubit™ dsDNA BR kit can be miniaturized to 1/10th of its volume while keeping good performance by using small volume 384-well microplates and the Tristar multimode readers.

Introduction

Most molecular biology methods require either normalizing all samples to the same quantity of starting DNA, or a specific input quantity. Thus, accurate DNA quantification is needed for techniques such as Next-Generation Sequencing (NGS), Polymerase Chain Reaction (PCR), Real-Time PCR (quantitative PCR; qPCR), cloning, transfection, and others.

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DNA quantification is performed very often using UV spectrophotometry. This method is simple, cost-effective and requires minimal sample quantity (typically 1 µL). However, it suffers from low specificity: it cannot distinguish between dsDNA and ssDNA or between DNA and RNA, or even between degraded and intact DNA. In addition, it is sensitive to the presence of contaminants that are frequent in DNA samples, such as protein, phenol or guanidine salts. Depending on the type of sample and downstream method to be used, it may be necessary to use methods of higher specificity. This is the case, for example, of NGS, which we already covered in a previous application note [1].

DNA quantification methods based on fluorescence offer a much higher specificity than UV spectrophotometry. They use intercalating dyes that are specific of the type of nucleic acid to be quantified and don't bind to free nucleotides. Qubit™ assay kits are a very popular example of fluorescence-based DNA quantification methods. While they are intended to be used by laboratories processing low numbers of samples and are performed in tube format, we have previously shown that they can be measured in 96-well plates with good performance using the Tristar multimode readers [1].

Moving from 0.5 mL tubes to 96-well microplates notably increases throughput, but moving to a microplate format with higher well count and smaller wells would allow to miniaturize the assay, providing even higher throughput and reducing reagent costs:

typical working volume are 200 µL in 96-well microplates, 75 µL in 384-well microplates and 20 µL in small volume 384-well microplates. Hence, moving from 96- to 384-well microplates would bring more than a 60% saving in reagent costs, a 90% if moving to small volume 384-well microplates.

In this Application Note we compare the performance of the Qubit™ dsDNA BR assay kit in 96-, 384- and small volume 384-well (384sv) microplates using the Tristar 3 and Tristar 5 multimode microplate readers.

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- Time-Resolved FRET (TR-FRET / HTRF®)
- Fluorescence Polarization
- AlphaScreen®
- Top and Bottom Reading
- Incubation

Materials

- Tristar 3 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69173-10).
- Tristar 5 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69185-15).
- Qubit™ dsDNA BR Assay Kit from ThermoFisher (Cat. # Q32850).
- Invitrogen™ Salmon Sperm DNA, sheared (10 mg/mL) from ThermoFisher (Cat. #AM9680).
- Black 96-well microplates from Berthold Technologies (Id. Nr. 23302).
- Black 384-well microplates from Greiner (Ref. 781076).
- Black small volume 384-well microplates from Greiner (Ref. 784900).
- Tubes (various volumes).
- Pipettes and pipette tips (various volumes).
- LightCompass® software from Berthold Technologies.

Instrument settings

- Reading mode: Fluorescence Endpoint
- Excitation filter: 485/14
- Emission filter: 535/25
- Counting time: 0.1 s
- All other settings with default values

Methods

Reagents were prepared following the manufacturer's instructions. In order to further extend the concentration range of the assay, a more concentrated DNA standard was used instead of the one included with the kit. The DNA stock was diluted to a concentration of 160 ng/µL using TE buffer. To prepare the standard curve, a ½ dilution series was prepared using this diluted stock, producing the following DNA concentrations: 80, 40, 20, 10, 5, 2.5, and 1.25 ng/µL DNA. TE buffer was used as blank.

In addition, to assess the performance of the assay, 3 "unknown" samples were prepared with concentrations different to that of the standard points: 120, 30 and 7.5 ng/uL DNA.

To minimize the differences between microplate formats due to sample handling, one single tube was prepared for all microplate formats and replicates of each standard or sample. 50 µL of standard or sample were mixed with 950 µL of Qubit working reagent in 1.5 mL microcentrifuge tubes and vortexed vigorously for 5 seconds. After incubating the tubes 5 minutes at room temperature protected from light, the following volumes were pipetted in triplicate in the wells of the corresponding microplates: 200 µL for 96-well microplates, 75 µL for 384-well microplates and 20 µL for 384sv microplates. Then, the microplate was measured in the Tristar reader using the settings described above. 4-parameter logistic (4PL) regression was used to calculate concentrations, as this algorithm provided the best results in previous tests [1].

Results

The standard curves produced were slightly curved in all cases (Fig. 1), in agreement with example results displayed in the User Guide of the Qubit™ assay [2] and with our previous results [1]. Again, when expressing measurements as relative fluorescence, both the Tristar 3 and the Tristar 5 produce very similar curves (Fig. 1 and 2). In both instruments, the curved shape is more accentuated in the case of 384sv microplates.

Results from a previous application note show that using standard curves with 5 or 4 points produces very similar results to the full 9-point standard curve [1]. Hence, curves with 5 and 4 standard points were used to calculate the concentrations of the unknown samples. The standard points chosen for each standard curve were the following ones:

5 points: 160, 40, 10, 2.5, 0 ng/µL
4 points "high": 160, 40, 5, 0 ng/µL
4 points "low": 160, 40, 1.25, 0 ng/µL

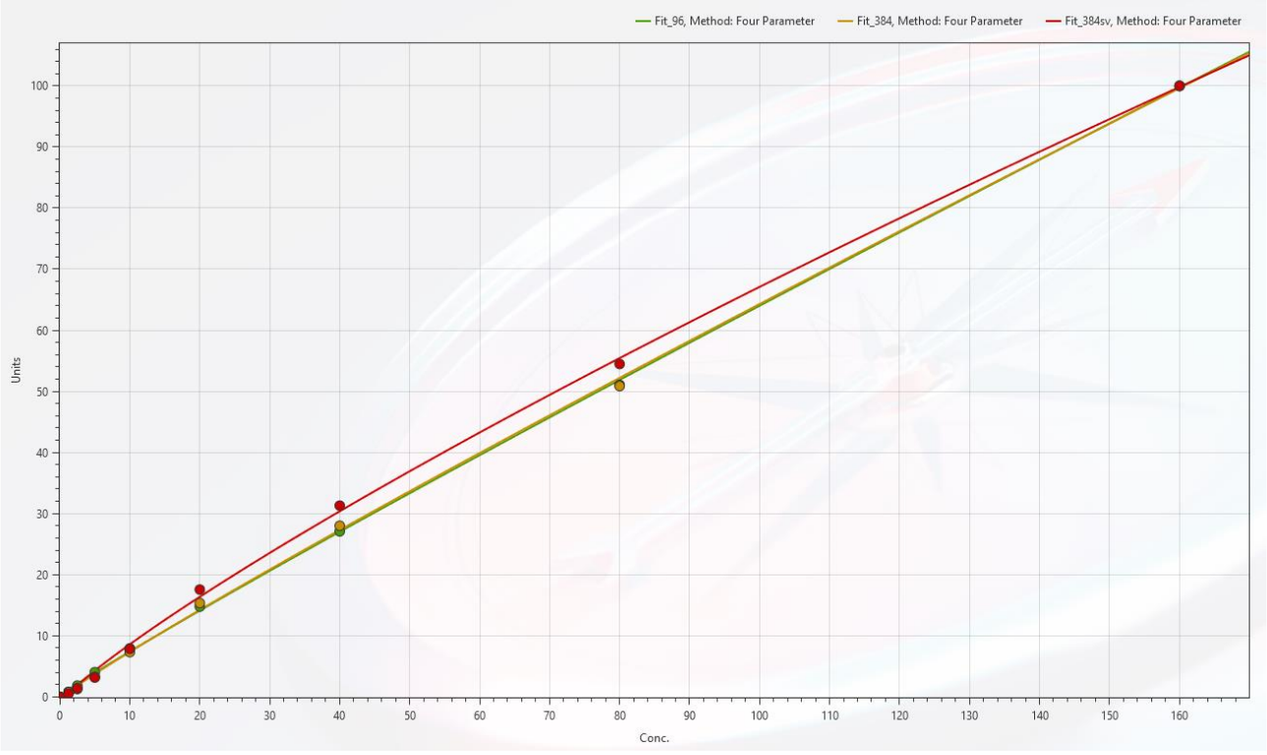

Figure 1. Standard curves generated by 4PL regression in the Tristar 5 in 96-, 384- and small volume 384-well (384sv) microplates. Measurement values expressed as % of the maximum fluorescence, concentrations in ng/µL. All data points measured in triplicat e.

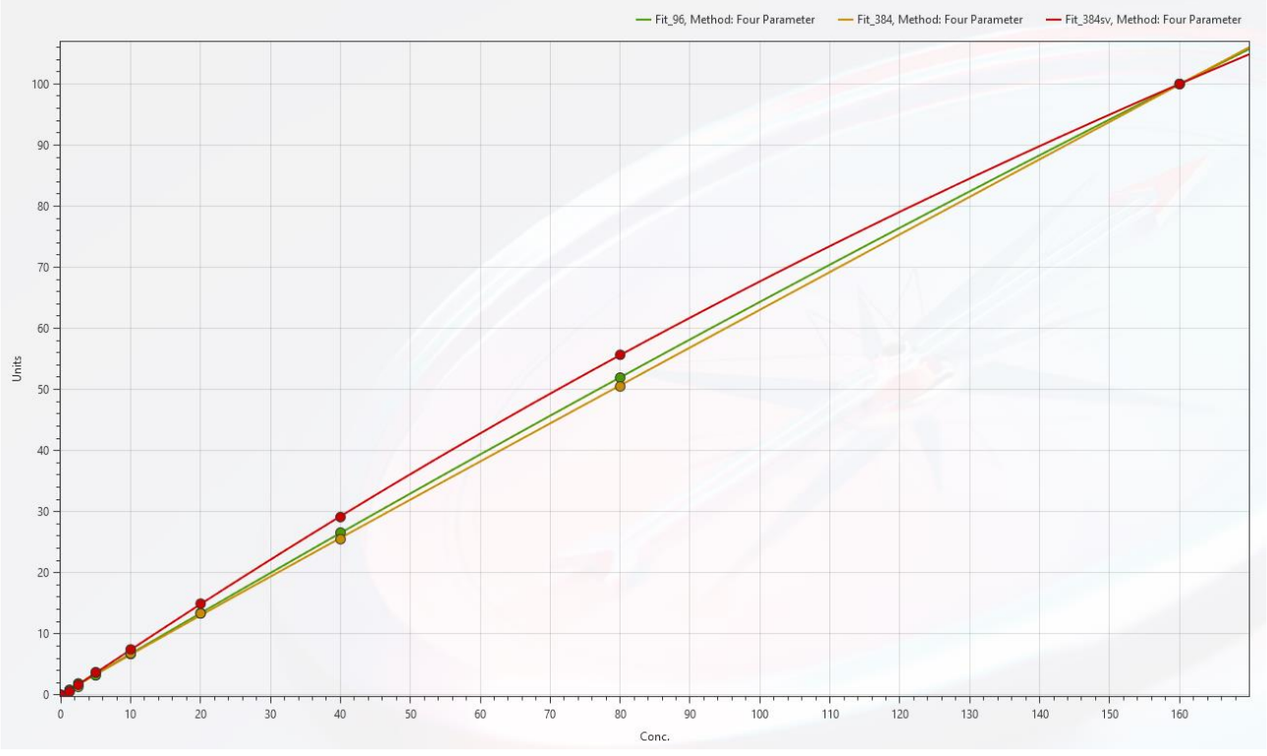

Figure 2. Standard curves generated by 4PL regression in the Tristar 3 in 96-, 384- and small volume 384-well (384sv) microplates. Measurement values expressed as % of the maximum fluorescence, concentrations in ng/µL. All data points measured in triplicat e.

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The accuracy of the quantification is different depending on the microplate format and the standard curve used. In 96-well microplates, both the 5-point and 4-point "high" standard curves deliver highly accurate results in both instruments (Table 1), with an average error below 2.5%. On the other hand, the accuracy is worse when using the 'low' curve. There are differences between the instruments tested for standard 384-well microplates: With the Tristar 5, 4-point curves give slightly better results than the 5-point curve, but the differences are comparatively small. With the Tristar 3, the best results are obtained with the 5-point standard curve. Finally, in small volume 384-well microplates, the best results in both instruments are obtained with the 5-point standard curve.

If only the results between both models of 384-well microplates are compared, it is remarkable that the accuracy in small volume is better than in standard microplates: in standard 384-well microplates, the curve providing the best results produces quantifications with an average error of 6.6% in the Tristar 5 (6.3% in the Tristar 3), while in small volume 384-well microplates the 5-point standard curve delivers an average error of quantification of only 3.2% and 4.9%, respectively.

Tristar 5	**96-well**			**384-well**			**384sv**		
ng/uL	5 points	4 points "high"	4 points "low"	5 points	4 points "high"	4 points "low"	5 points	4 points "high"	4 points "low"
120	116.11	116.27	114.57	115.24	114.10	114.10	125.85	123.85	122.98
30	28.86	29.06	29.58	31.55	31.82	31.81	30.59	31.07	31.25
7.5	7.53	7.59	8.49	6.49	6.85	6.85	7.70	8.55	8.94
Tristar 3	**96-well**			**384-well**			**small volume 384-well**		
ng/uL	5 points	4 points "high"	4 points "low"	5 points	4 points "high"	4 points "low"	5 points	4 points "high"	4 points "low"
120	117.78	117.03	117.76	120.49	120.59	122.98	128.06	128.01	125.01
30	29.10	29.28	29.09	27.48	27.41	31.25	27.78	27.81	28.70
7.5	7.29	7.67	7.34	6.76	6.48	8.94	7.54	7.53	8.99

Table 1. Calculated dsDNA concentrations using 4PL algorithm and different standard curves. All concentrations in ng/µL. All data points measured in triplicate.

Discussion and conclusions

Adapting the Qubit™ dsDNA BR assay kit to 96-well microplate format increases throughput while delivering very good accuracy in both Tristar multimode readers with a standard curve of only 4 points. Switching to 384-well microplates further increases throughput and, in addition, reduces the amount of reagent needed for the quantification. Accuracy in 384-well microplates is lower than in 96-well format but, when choosing the right microplate and standard curve, accuracy loss is small: in the Tristar 5 average error increases from 2.5% in 96-well microplates to only a 3.2% in 384sv microplates using the 5-point standard curve, a 4.9% in the Tristar 3. In many cases, this loss in accuracy is more than offset by the savings and increase in throughput.

It is remarkable that performance is worse in standard 384-well microplates than in small volume ones in both instruments. A possible factor is that wells are square in standard microplates but round in small volume ones. Determining the origin of this differences is beyond the scope of this application note but, from a practical perspective, any laboratories considering moving to 384-well microplates should choose small well ones, as they provide not only the highest reagent savings, but also the best performance compared to standard 384-well microplates.

The best experimental setup for each microplate format for the concentration range tested is summarized in table 2:

	96-well	384-well	384sv
µL/well	200	75	20
Algorithm	4PL	not recommended	4PL
Standard points	4		5
Standard concentrations (ng/µL)	160, 40, 5, 0		160, 40, 10, 2.5, 0

Table 2. Optimal experimental settings for each microplate format.

A final thought on the concentration range: the specific concentrations for the standard curve used in this application note are arbitrary, and laboratories can choose different concentration ranges depending on their needs and the expected concentrations of their samples (for example, up to 100 ng/µL only, to make use of the DNA standard included with the kit).

In this application note we demonstrate that the Qubit™ dsDNA BR assay can be miniaturized, reducing total reaction volume from 200 µL down to 20 µL, using the Tristar multimode readers and small volume 384-well microplates. Using a 4-parameter logistic algorithm, 5 standard points are sufficient to provide good performance in the concentration range tested: this allows 187 samples to be quickly quantified in duplicate (123 samples in triplicate) in a single 384-well plate and using only 7.3 mL of Qubit™ working reagent instead of the 18.2 mL needed in 96-well format. For laboratories working with low-concentration samples, the Qubit™ dsDNA HS assay kit is available; we expect it to also work very well in microplate format using the Tristar multimode readers.

Taking everything into account, the Tristar multimode readers are a very good solution to miniaturize the Qubit™ dsDNA BR assay. Using the curve fitting algorithms and standard points detailed in this application note, they offer a DNA quantification solution with low running costs and high reliability and throughput. With the adjustments we have made to the protocol, it will be a valuable tool for laboratories using Qubit™ assays for DNA quantification in large quantities of samples.

References

1. Quantifying DNA for NGS with the Qubit™ dsDNA BR kit and Tristar multimode readers (2024). Berthold Technologies. Available for download at www.berthold.com.
2. User Guide of the Qubit™ dsDNA BR Assay Kit (2022). Pub. No. MAN0002325, Rev. B0. Thermo Fisher Scientific.

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