

## Application Note

# QIAxpert® QIASymphony® Spectral Content Profiling App

Daniel Lehmann, Katharina Pfeifer-Sancar, Marion Egli, Carola Schade

QIAGEN GmbH

## Introduction

A new analysis protocol (App) for precise quantification of mammalian DNA is available for the QIAxpert spectrophotometer. The QIAxpert DNA QIASymphony App is for quantification of double-stranded DNA purified using QIASymphony and EZ1® technology.

The proprietary spectral content profiling is used to separate the profile of the QIASymphony purified DNA from the measured UV/VIS spectrum, thereby distinguishing it from interfering chemicals and other potential contaminants. The specific DNA profile is then used to determine the DNA concentration (ng/µl) based on the absorbance at 260 nm using a concentration factor of 50.

In this Application Note, we describe how the App presents data and show data on performance of the QIAxpert DNA QIASymphony App.



Figure 1. The QIAxpert DNA QIASymphony App icon.

## Materials and Methods

### System requirements and compatibility

The DNA QIASymphony App is identified by the icon shown in Figure 1. Compatible kits are listed in Table 1.

Table 1. Kits compatible with the QIAxpert DNA QIASymphony App\*

Product	Contents	Cat. no.
QIASymphony DNA Mini Kit	For 192 preps of 200 µl each: Includes 2 reagent cartridges and enzyme racks and accessories.	931236
QIASymphony DNA Midi Kit	For 96 preps of 1000 µl each or 144 preps of 400 µl each: Includes 2 reagent cartridges and enzyme racks and accessories.	931255
EZ1 DNA Blood 200 Kit	For 48 preps of 200 µl each: Includes reagent cartridges and accessories.	951034
EZ1 DNA Blood 350 Kit	For 48 preps of 350 µl each: Includes reagent cartridges and accessories.	951054
EZ1 DNA Tissue Kit	For 48 preps of up to 40 mg samples each: Includes reagent cartridges and accessories.	953034

\* The applications presented here are for molecular biology applications. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

## Profile description

The spectral content profiling algorithms in this App differentiates RNA, DNA and other impurities, as well as background of the sample by analyzing the full measured UV/VIS spectrum (white curve) and provides distinct profiles as a result (Figure 2):

- DNA shown as the blue curve – is the molecule of interest. This profile is specific for dsDNA (40–45% GC).
- Impurities (orange curve) – non-DNA molecules that also absorb in the UV region (e.g., RNA residue, thiocyanate salts, EDTA, citrate and azide).
- Background (grey curve) – profile combining intrinsic sample turbidity, scattering from bead contamination or other particles, as well as hemoglobin/heme residue (if present, an absorbance at approximately 410 nm is visible). This background spectrum is subtracted from the measurement prior to profiling.
- Residue (yellow curve) – unidentified part of the measured spectrum, also shown as a percentage value of the measured spectrum (white curve).

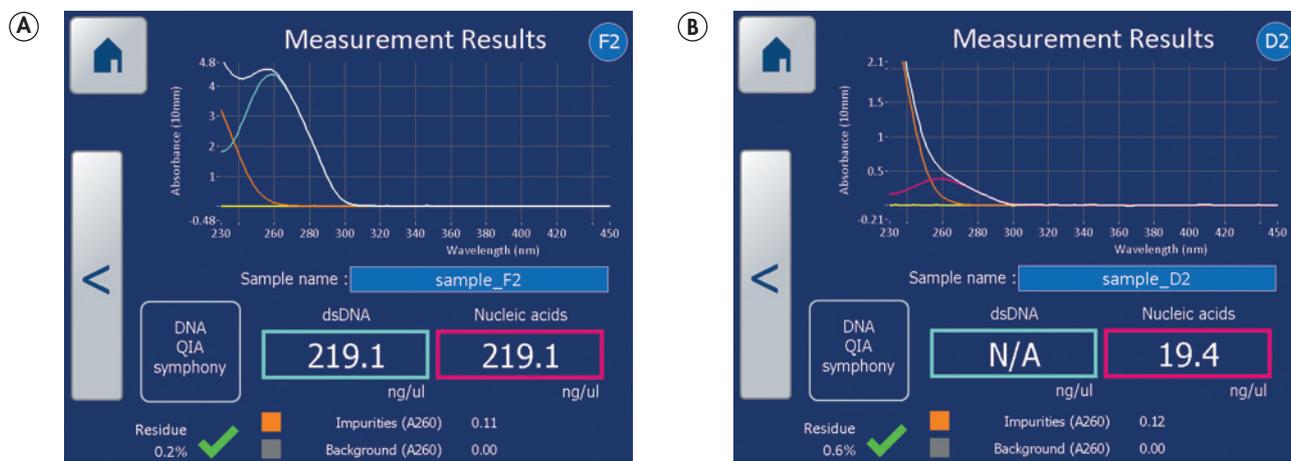
Spectral profiling of dsDNA is unavailable in specific circumstances for samples with:

- $A_{260}$  below 0.5 OD (10 mm) after background correction; i.e., if the nucleic acid concentration of the sample is below 25 ng/μl (Figure 2B)
- residue above 2.5% due to an unknown component (a red cross instead of a green checkmark is shown next to the residue value)

In these circumstances, a total nucleic acids spectrum (pink curve) is displayed instead of the DNA profile. The concentration is calculated using the  $A_{260}$  value of this profile multiplied by the concentration factor of dsDNA (i.e., 50).

## Blanks

For spectral content profiling analysis, sample elution buffer as a blank is not required and will adversely affect the result. Required corrections for spectral content profiling are performed via an automatic blanking by the system. Alternatively, pure water (ddH<sub>2</sub>O) can be used as the blank. For recommendations on blanks, see the *QIAxpert User Manual* (available at [www.qiagen.com/qiaexpert](http://www.qiagen.com/qiaexpert)).



**Figure 2. Example result screen for spectral content analysis.** Mammalian DNA was purified using the QIAAsymphony DNA DSP Mini Kit and the QIAxpert DNA QIAAsymphony App was used for measurement. **A.** A DNA-profiled sample with a DNA concentration of 219.1 ng/μl and a low residue value of 0.2%. **B.** A DNA sample, where DNA profiling is unavailable, due to a low nucleic acid concentration of 19.4 ng/μl, but still showing a low residue value of 0.6%.

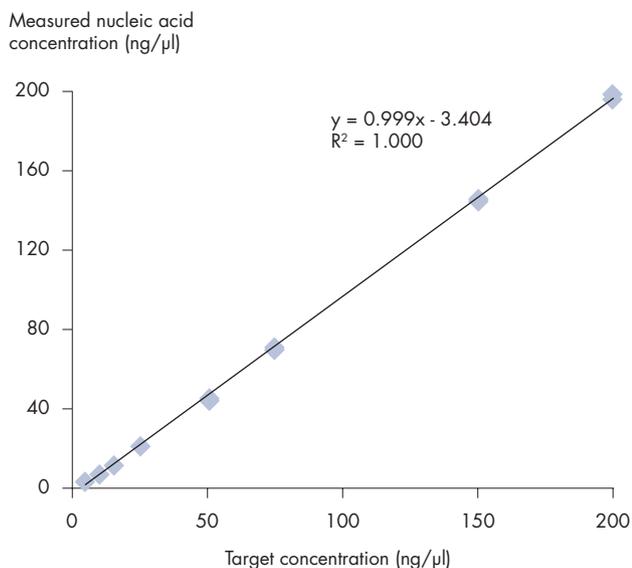
## Results

### Performance data

The performance of the DNA QIASymphony App was determined regarding linear regression: measured as target concentration vs. measured concentration and precision/reproducibility standard deviation (STD [ng/μl]) of multifold measurement of one sample.

Linearity and precision/reproducibility characteristics of the DNA QIASymphony App were determined by measuring a dilution series of calf thymus DNA (purified using the QIASymphony instrument and QIASymphony DNA DSP Mini Kit). Concentrations of calf thymus DNA ranging from 200 ng/μl to 5 ng/μl were prepared in buffer ATE. Each concentration was measured 5 times on the same QIAxpert instrument using the DNA QIASymphony App with the automatic blanking option.

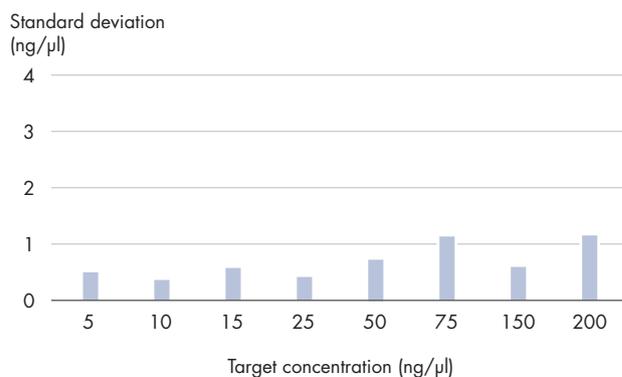
Results showed a high linearity with a calculated regression coefficient of  $R^2 = 1.000$  (Figure 3).



**Figure 3. Linear regression of dilutions of purified calf thymus DNA.** Each concentration was measured 5 times using QIAxpert DNA QIASymphony App.

Precision and reproducibility of the QIAxpert DNA QIASymphony App was evaluated for the 5 repeated measures with concentrations from 200 ng/μl down to 5 ng/μl. STD (ng/μl) was calculated for each concentration as a measure of precision.

Variability within the 5 repeated measurements for each dilution was low, with STD (ng/μl) below 1 ng/μl for the majority of concentrations tested. Only some of the higher concentrations (75 ng/μl and 200 ng/μl) showed a STD slightly above 1 ng/μl (Figure 4).



Target concentration (ng/μl)	STD (ng/μl)
5	0.48
10	0.36
15	0.58
25	0.42
50	0.72
75	1.15
150	0.60
200	1.15

**Figure 4. STD (ng/μl) of dilution-series measurements of purified calf thymus DNA.** The STD (ng/μl) was determined for each 5-fold measured concentration. The measurements were recorded on the same QIAxpert instrument using QIAxpert DNA QIASymphony App.

## Conclusion

The QIAxpert DNA QIASymphony App shows excellent performance in terms of linearity and variability with a limit of detection of 1.5 ng/μl.

The App enabled precise quantification of mammalian gDNA purified by the bead-based QIASymphony system (see Table 1 for compatible kits).

Overall, the QIAxpert performs nucleic acid quantification and quality control in a fast and easy manner, analyzing up to 16 samples within 2 minutes. Furthermore, the use of microfluidic QIAxpert slides provides qualitative measurements by avoiding cross-contamination, as well as evaporation or meniscus effects.

## Download Information

Product	Contents	Link
QIAxpert DNA QIASymphony App	For quantification of dsDNA purified with QIASymphony technology: the concentration (ng/μl) is determined based on the absorbance at 260 nm using a concentration factor of 50.	<a href="http://www.qiagen.com/qiexpert">www.qiagen.com/qiexpert</a>

The applications presented here are for molecular biology applications. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. It is the user's responsibility to validate the performance of the protocol with QIAxpert DNA QIASymphony App for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. The performance characteristics of this product have not been fully established.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at [www.qiagen.com](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

See how you can benefit from QIAxpert at [www.qiagen.com/qiexpert](http://www.qiagen.com/qiexpert).

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