Application Note

QIAxpert® Spectral Content Profiling Protocols

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Introduction

Determining the purity and concentration of nucleic acid samples is an essential application in molecular biology today, especially for complex workflows. Two of the greatest challenges are ensuring that each nucleic acid sample has an adequate and accurate concentration and that any chemical contaminants or other impurities are detected.

With classic UV/Vis spectrophotometric measurements, the concentration calculation relies only on the absorbance at 260 nm. Since the method is not very selective and sensitive, all nucleic acids as well as other contaminants in the sample can contribute to this result, failing to distinguish your biomolecule of interest from other artifacts. This may lead to overestimation of the actual DNA or RNA concentration and jeopardize your downstream analysis.

Using a fluorescent dye-based method can help quantify only the molecule of interest, but such a method is unable to provide detailed information regarding the presence of contaminants or detect the presence of another nucleic acid within the same measurement.

QIAxpert spectral profiling combines the advantages of both methods and ensures sample quality and quantity assessment that is reliable. The smart analysis algorithm allows easy differentiation between DNA, RNA, and other contaminants within one, dye-free measurement run. By reporting total nucleic acid and molecule of interest concentration separately and by detecting and subtracting concentration of contaminants, QIAxpert gives true insight into your sample composition (Table 1).

Table 1. Nucleic acid quality control parameters

QC parameter	Classic Spectrophotometry	Dye-based Fluorometry	QIAxpert Spectrophotometer	QIAxcel Advanced System*
Concentration	•	•	•	•
Protein contaminants (A_{260}/A_{280})	•		•	
Salts & other contaminants (A_{260}/A_{230})	•		•	
Differentiation between DNA, RNA, impurities			•	
Size range & degradation				•

* Fully automated gel electrophoresis

Measurement Principle

QIAxpert spectrophotometer offers two measurement modes to accommodate all nucleic acid sample types, classic UV/Vis protocols as available on any spectrophotometer (Figure 1A) and unique spectral content profiling protocols that unmix the spectra and discriminate between DNA, RNA and impurities (Figure 1B). Refer to the tables ahead for further information on available protocols.

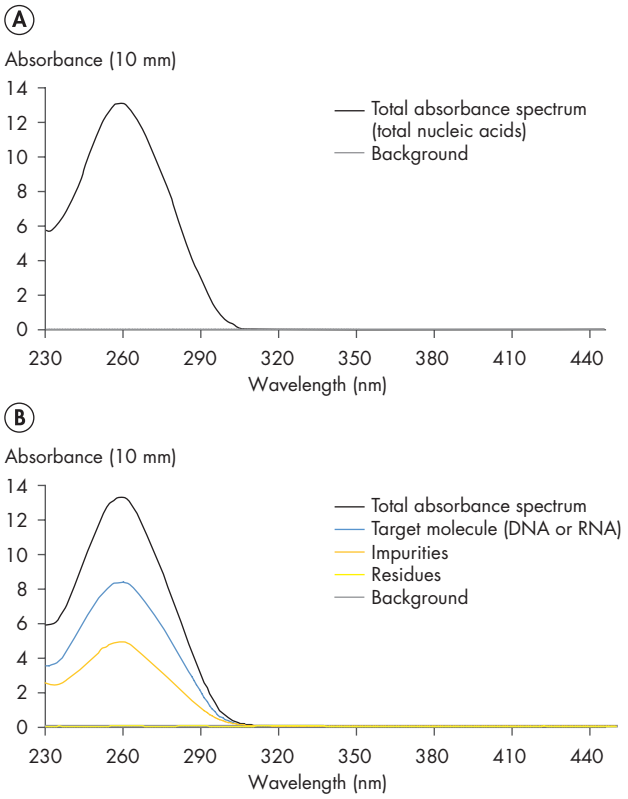


Figure 1. Comparison of methods used for determining nucleic acid quantity and purity. A Classic UV/Vis spectrophotometry. **B** UV/Vis spectrophotometry with spectral profiling.

These apps use state-of-the-art software algorithms to extract the contribution of specific components in a mixture from the measured UV/Vis spectrum. The principle is based on Beer's law for mixtures, which states that the absorption spectrum of a mixture is a linear combination of the spectra of the constituents.

Using reference spectra, the inverse solution of decomposing a measured spectrum in a linear combination of spectra originating from its constituents is used to determine concentrations of components in a mixture. The contribution of the profile of the molecule of interest (e.g., DNA or RNA), together with the residual profile of impurities and sample turbidity, can be accurately determined within the recorded spectrum.

QIAxpert relies on high-speed microfluidic UV/Vis technology which unlocks several excellent benefits for spectrophotometry measurement (Figure 2). QIAxpert can analyze up to 16 samples in less than 2 minutes and enables rapid discrimination between sample components, contaminant detection and accurate concentration measurements of microvolumes of nucleic acids.

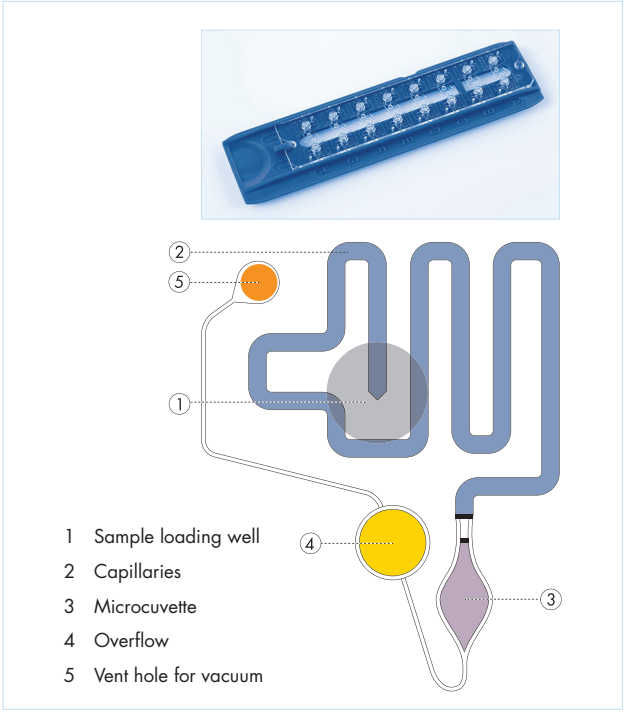


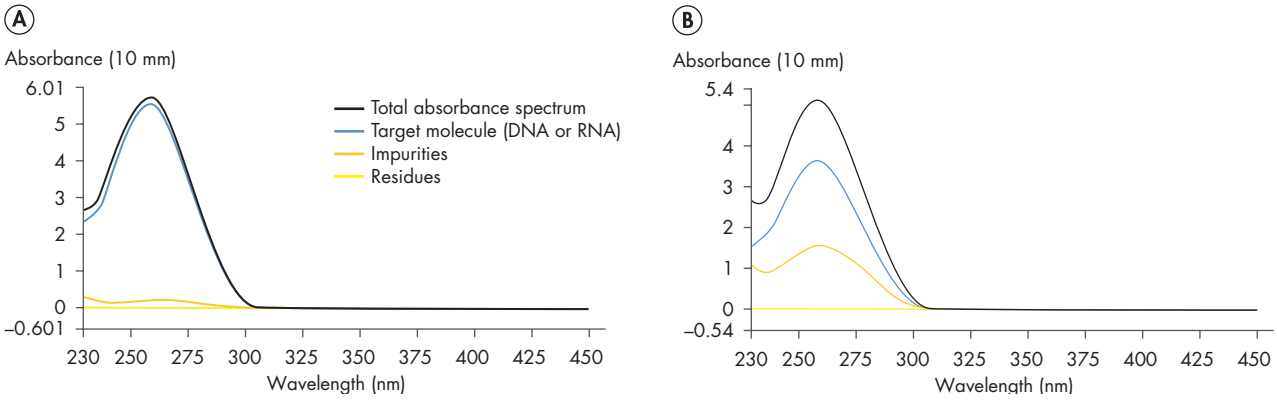
Figure 2. Microfluidic features of the QIAxpert slide

Blanking recommendations

Using sample elution buffer as blank is not recommended for spectral content profiling analysis as it can adversely affect the result. Required corrections for spectral content profiling are performed via an automatic blanking done by the system. Alternatively, pure water (ddH_2O) can be used as blank. For classic UV/Vis measurement, use sample buffer as blank.

For further recommendations on blanks, see the corresponding *QIAxpert User Manual* available at **www.qiagen.com/qiaxpert**.

Data Example



Spectral content profiling discriminates between components in complex samples. A A pure sample of calf thymus DNA shows no significant contamination, as evidenced by the simple spectrum. **B** A sample of DNA spiked with RNA was precisely analyzed and quantified. Both samples were measured on the QIAxpert DNA QIAamp application. DNA is indicated by the blue absorbance line while RNA and all detected impurities are depicted by the orange line. A gray line typically appears as a result of the sample background spectrum. Due to low sample background in this case, the gray line is not visible. A yellow line typically depicts the residual spectrum that cannot be attributed to reference profiles used in the algorithm. In this case, the yellow line is flat because no residual components could be detected. The sum of nucleic acid content, impurity and residual spectrum is represented by the black line.

Read the full Application Note at **www.qiagen.com/QIAxpert-QC**.

Available Spectral Content Profiling Protocols (Apps)

QIAGEN Purification Kits-specific Apps

Specific spectral profiling applications ("apps") have been designed for nucleic acids that have been purified using QIAGEN chemistries. The apps are thoroughly verified and validated by QIAGEN and assures delivery of most reliable results.

App Name	Description/Use	Icon
DNA QIAamp	Mammalian genomic DNA (gDNA) purified with QIAamp® technology	
DNA QIAsymphony	Mammalian genomic DNA (gDNA) purified with QIAsymphony® or EZ1® technology	
PCR QIAquick	PCR amplicons purified with QIAquick® technology	
RNA RNeasy	Total RNA purified with RNeasy® technology	
RNA PAXgene	Human whole blood RNA purified with PAXgene® Blood RNA technology	

Note: Check compatibility list on **www.qiagen.com/qiaxpert** and get a full overview on compatible kits.

General Apps

For non-QIAGEN purification chemistry or for samples where no specific app is available.

Note: If the residual value in the result is >2.5%, spectral content profiling will not be displayed. What will be displayed as residues is absorbing material that cannot be attributed to known components of the profile.

App Name	Description/Use	Icon
DNA Mamm.	Used for spectral content profiling + specific quantification (ng/µl) of human/ mammalian dsDNA (40–45%GC). Sample background and impurities are quantified as A_{260} values	
DNA Plant	Used for spectral content profiling + specific quantification (ng/µl) of plant dsDNA (30–50%GC). Sample background and impurities are quantified as A_{260} values	
Purified PCR	Used for spectral content profiling + specific quantification (ng/µl) of the dsDNA amplicon. Sample background and impurities are quantified as A_{260} values	
RNA	Used for spectral content profiling + specific quantification (ng/µl) of RNA isolated from any source. Sample background and impurities are quantified as A_{260} values	
RNA FFPE	Used for spectral content profiling + specific quantification (ng/µl) of RNA from FFPE specimens. Sample background and impurities are quantified as A_{260} values	

Classic UV/Vis Apps

Classic UV/Vis apps are also available on the QIAxpert for samples where no specific app is available, or no spectral content profiling is needed.

App Name	Description/Use	Icon
A_{260} dsDNA	Used for quantification of dsDNA based on the total absorbance at 260 nm, the concentration (ng/µl) is determined using a concentration factor of 50	A260 dsDNA
A_{260} ssDNA	Used for quantification of ssDNA based on the total absorbance at 260 nm, the concentration (ng/µl) is determined using a concentration factor of 33	A260 ssDNA
A_{260} RNA	Used for quantification of total RNA based on the total absorbance at 260 nm, the concentration (ng/µl) is determined using a concentration factor of 40	A260 RNA
A_{280} Protein	Used for quantification of purified proteins based on the total absorbance at 280 nm, tap to the next screen to enter E1% extinction coefficients for concentration (mg/ml) determination	A280 Protein
UV/Vis	Used for conventional full UV/Vis spectral measurements, with selection of up to 3 wavelengths for absorption measurement	UV/vis
OD check	Validation of photometric accuracy with a potassium dichromate ($K_2Cr_2O_7$) solution	OD check

Software Download Information

Product	Contents
SW 2.4.0.59	Updated application software containing new spectral content profiling applications. Includes the following QIAxpert spectral content profiling applications to be used with QIAGEN chemistry: PCR QIAquick v.2.4.0.1, RNA RNeasy v.2.4.0.9, DNA QIAamp v.2.4.0.9, DNA QIAsymphony v.2.4.0.2, and PAXgene RNA v.2.4.0.0. And also the following spectral content profiling applications to be used for samples where no specific app is available: DNA Mamm. v.2.4.0.1, DNA Plant v.2.4.0.1, Purified PCR v.2.4.0.1, RNA v.2.4.0.3, RNA FFPE v.2.4.0.1. Refer to the *QIAxpert User Manual* for software installation instructions.

Note: The software can be downloaded from **www.qiagen.com/qiaxpert** under the Resource tab.

Results and Reporting Options

QIAxpert offers more flexibility in terms of data reporting (Figure 3). Results are easily exported to an USB stick or a smart device. QIAxpert thus provides a unique combination of features that make it an attractive alternative to other nucleic acid analysis systems on the market.

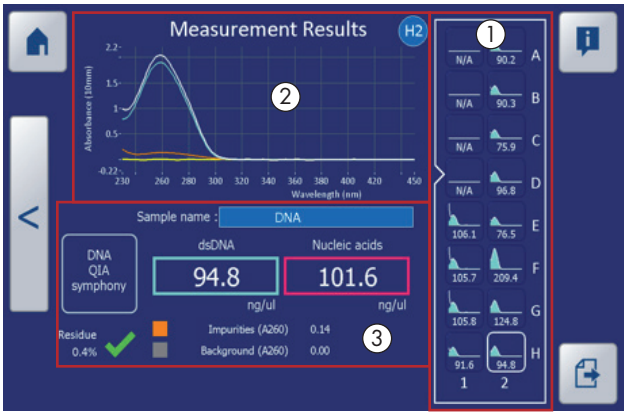


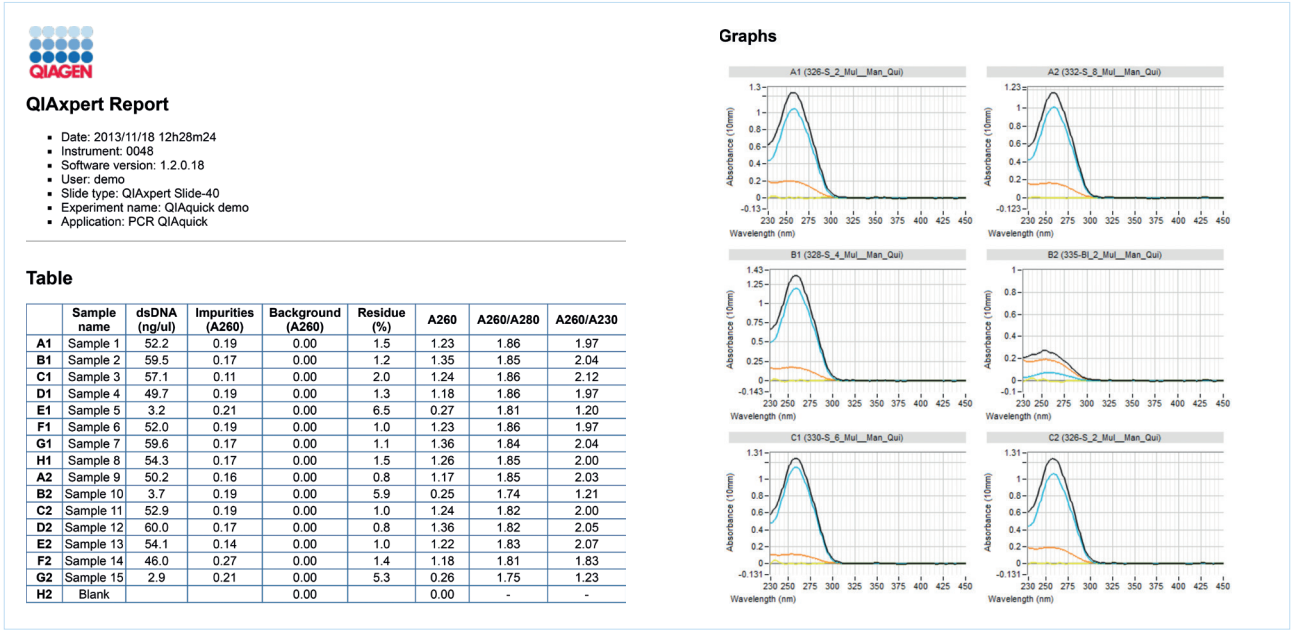
Figure 3. Measurement results screen.

1 **Slide overview**
2 **Details of spectral curve for activated well**
3 **Sample results for activated well**

Spectral curve color codes:

White:	Pure UV/Vis spectrum of sample
Blue:	Spectrum of target molecule (DNA App = DNA; RNA App = RNA)
Orange:	Spectrum of impurities (mainly kit components that are known by the algorithm; DNA in RNA App and vice versa)
Yellow:	Spectrum of residues (mathematical difference between measured and fitted; e.g., absorbing molecules that are unknown by the algorithm)
Grey:	Background (system-related corrections, turbidity, beads, hemoglobin)

QIAxpert automatically generates a comprehensive .html report that can be viewed in any browser and is exported automatically with other result files such as .csv, .txt, and .xml formats (Figure 4). ▷



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QIAxpert Report

- Date: 2013/11/18 12h28m24
- Instrument: 0048
- Software version: 1.2.0.18
- User: demo
- Slide type: QIAxpert Slide-40
- Experiment name: QIAquick demo
- Application: PCR QIAquick

Table

	Sample name	dsDNA (ng/ul)	Impurities (A260)	Background (A260)	Residue (%)	A260	A260/A280	A260/A230
A1	Sample 1	52.2	0.19	0.00	1.5	1.23	1.86	1.97
B1	Sample 2	59.5	0.17	0.00	1.2	1.35	1.85	2.04
C1	Sample 3	57.1	0.11	0.00	2.0	1.24	1.86	2.12
D1	Sample 4	49.7	0.19	0.00	1.3	1.18	1.86	1.97
E1	Sample 5	3.2	0.21	0.00	6.5	0.27	1.81	1.20
F1	Sample 6	52.0	0.19	0.00	1.0	1.23	1.86	1.97
G1	Sample 7	59.6	0.17	0.00	1.1	1.36	1.84	2.04
H1	Sample 8	54.3	0.17	0.00	1.5	1.26	1.85	2.00
A2	Sample 9	50.2	0.16	0.00	0.8	1.17	1.85	2.03
B2	Sample 10	3.7	0.19	0.00	5.9	0.25	1.74	1.21
C2	Sample 11	52.9	0.19	0.00	1.0	1.24	1.82	2.00
D2	Sample 12	60.0	0.17	0.00	0.8	1.36	1.82	2.05
E2	Sample 13	54.1	0.14	0.00	1.0	1.22	1.83	2.07
F2	Sample 14	46.0	0.27	0.00	1.4	1.18	1.81	1.83
G2	Sample 15	2.9	0.21	0.00	5.3	0.26	1.75	1.23
H2	Blank			0.00		0.00	-	-

Graphs

Figure 4. QIAxpert .html report with experiment details, results table and spectra.

Summary

Novel UV/Vis spectrophotometers such as the QIAxpert System enable fast and accurate nucleic acid quantification, while eliminating the need for repetitive drop-and-clean actions, thus minimizing the risk of cross-contamination and streamlining the workflows in your lab. In addition, unique spectral protocols overcome the challenges posed by conventional methods, allow discriminating between molecules of interest and provide researchers with a more comprehensive understanding of sample composition. Gaining true insight into their samples, researchers can then make better-informed decisions with regard to usage of those samples in their subsequent downstream processes.

Find out more on how you can benefit from the QIAxpert at **www.qiagen.com/qiaxpert.**

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 PAXgene RNA App for any particular application, 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** or can be requested from QIAGEN Technical Services or your local distributor.