Technical Information

QIAxpert® Spectral Content Profiling

Rapid, accurate and dye-free quantification and quality control of nucleic acids – in a single measurement

Characterization of nucleic acid and protein samples is a quality control measure that should be adopted by every molecular and biochemistry lab. It encompasses quantification and detection of impurities present in nucleic acid samples, ranging from proteins to salts and organic solvents. The presence of co-purified substances (like polyphenolic metabolites, polysaccharides, hemoglobin or chlorophyll), carry-over from the isolation buffers, such as chaotropic salts or phenol, as well as turbidity effects caused by cell debris or residual magnetic beads can have a profound effect on the sensitivity and efficiency of downstream assays. Accurate and timely assessment of sample yield and purity can help you make well-informed decisions regarding whether a sample is suitable for downstream processing, or whether further refinement is necessary in order to ascertain the reliability of the resulting data [1, 2].

Limitations of current technologies for nucleic acid measurements

Although numerous analysis techniques exist, few are as fast, accurate and simple as UV/Vis spectrophotometry and fluorometry. Moreover, these other techniques have certain limitations as indicated in Table 1.

Spectrophotometric measurements enable calculation of nucleic acid concentrations based on a sample's absorbance at 260 nm. However, classical spectrophotometers also have limitations. Simply relying on this principle, they cannot differentiate between the types of nucleic acids as both DNA and RNA in the sample will contribute to the absorbance value at 260 nm, leading to incorrect assumptions regarding the starting sample quantity and purity [3]. Furthermore, overestimating the nucleic acid concentration due to the presence of other UV-absorbing contaminants contributing to the absorbance at 260 nm can negatively impact the reaction performance [4].

The absorbance values at 280 nm and 230 nm are widely used to compute the A_{260}/A_{280} and A_{260}/A_{230} ratios to assess the level of contaminating proteins or salts, respectively. These ratios provide an estimation of the sample purity, and can be used as an acceptance criteria for inclusion or exclusion of samples in downstream applications. However, the sensitivity and reliability of these assessments are a rising concern as reports demonstrate that even minor alterations in buffer ▷

composition used in these spectrophotometric evaluations can significantly affect these ratios, i.e., the ratios obtained do not always correlate with the actual sample contamination levels [3, 5].

Fluorometric measurements using specific fluorescent dyes help accurately quantify the molecule of interest from a sample containing a complex mixture of components. Fluorescent dye-based methods are generally more sensitive than absorbance-based methods, but are more time and resource intensive, as they involve additional steps for incubating samples with the fluorescent dyes and creating standard curves with known quantities of nucleic acid. Moreover, since the dye binds specifically to either double- or single-stranded DNA or RNA, the concentration calculated using these methods is dependent on the binding efficiency of the dyes and can lead to incorrect assumptions regarding total and target nucleic acids in the samples. Additionally, a disadvantage of fluorescence measurements is that they provide no information regarding sample impurities (Table 1).

Importance of QIAxpert spectral content profiling

The QIAxpert system is QIAGEN's next-generation spectrophotometer that combines the advantages of both classic spectrophotometry and fluorometry, while overcoming their limitations (Table 1). This next-generation spectrophotometry applies spectral content profiling subsequent to a classical UV/Vis measurement, determining the entire measured spectrum and differentiating between DNA, RNA and residual impurities. Spectral content profiling applications on the QIAxpert enable rapid, accurate and dye-free quantification of nucleic acid in solution containing a complex mixture of multiple UV/Vis-absorbing molecules [6]. The QIAxpert system works on smart analysis algorithms that separate the spectra and fit reference sample and buffer spectra, letting users focus on their molecule of interest [7].

Table 1. Comparison of various nucleic acid assessment technologies based on key measurement parameters

	UV/Vis Spectrophotometry	UV/Vis Spectrophotometry + Spectral Content Profiling	Fluorometry*
Limit of detection (order of magnitude)	ng/µl	ng/µl	pg/µl
Purity check	+	+	
Content profiling		+	
Differentiate RNA from DNA		+	+
Robust detection of contaminants		+	
No assay requirement	+	+	

* Requires a specific dye-based assay.

How UV/Vis spectral content profiling works and how to interpret the results

Spectral content profiling (SCP) uses state-of-the-art software algorithms to unravel the measured spectra into the relevant molecular profiles that contribute to the absorption. Unique spectral protocols enable powerful and reliable characterization of complex samples, wherein individual

components might have completely or partially overlapping absorbance profiles. Specific spectral profiling applications on the QIAxpert have been designed to cover challenging laboratory samples that may carry contaminants from purification workflows, e.g., phenol and salts, or samples containing co-extracted impurities (e.g., hemoglobin, proteins, cell debris and chlorophyll) [6].

The basic principle of SCP relies on the Beer-Lambert law for mixtures, which states that the absorption spectrum of a mixture is a linear combination of the spectra of its individual constituents [1, 6]. The reference spectra of RNA, DNA, proteins and a number of impurities are included in the pre-installed protocols ("apps") and serve as the basis of a mathematical model that deconvolutes the recorded spectrum and extracts the spectral contribution of specific components from the measured UV/Vis spectrum (Figure 1).

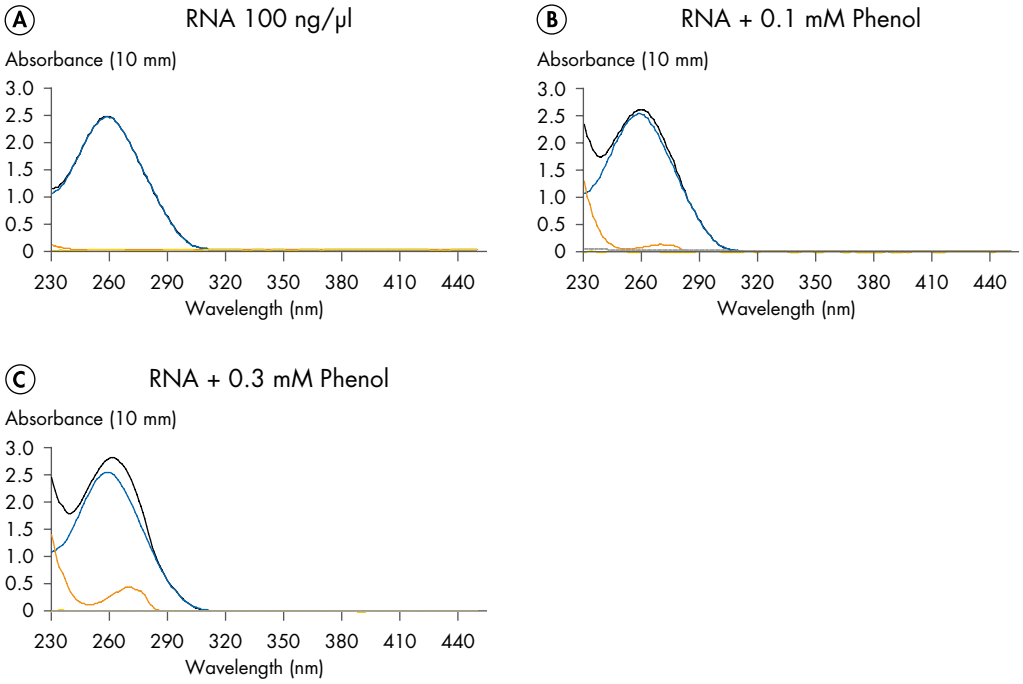

Figure	Sample	Total absorbance (A_{260})	Target RNA concentration (ng/µl)	Measured RNA concentration (ng/µl)	Impurities (A_{260})
A	Pure RNA	2.63	100	104.9	0.00
B	RNA + Phenol 0.1 mM	2.61	100	101.1	0.08
C	RNA + Phenol 0.3 mM	2.82	100	101.8	0.27

Figure 1. QIAxpert measurements with the RNA RNeasy App. RNA concentration and purity were measured on the QIAxpert with and without spiked-in phenol contamination. **Top** Spectral content profiling showing total measured spectrum (**black**), RNA spectrum (**blue**), impurities spectrum, including phenol (**orange**), residues (**yellow**; not visible, because no unknown components were detected) as well as background (**gray**; not visible due to low sample background). **Bottom** A table showing total absorbance values (A_{260}), target RNA concentrations (ng/µl), measured RNA concentrations (ng/µl) and the impurities values (A_{260}). These results demonstrate similar RNA concentration measurements (blue curve and the corresponding values) despite increasing phenol contamination (orange curve and the corresponding impurities values).

RNA and DNA molecules differ in their: i) carbohydrate backbone composition; where RNA has a ribose sugar and DNA has a deoxyribose sugar and ii) nucleotide content; as DNA molecules are heteropolymers of Adenosine, Cytosine, Guanine and Thymine, while RNA molecules have Uracil residues incorporated in place of Thymine [4]. Due to these structural differences, RNA and DNA also have slightly different absorption spectra in the UV/Vis spectrum and SCP algorithms can specifically detect and quantify DNA from RNA in a multicomponent system (Figure 2). The QIAxpert allows dye-free discrimination of these two nucleic acid molecules [6, 7, 8, 9].

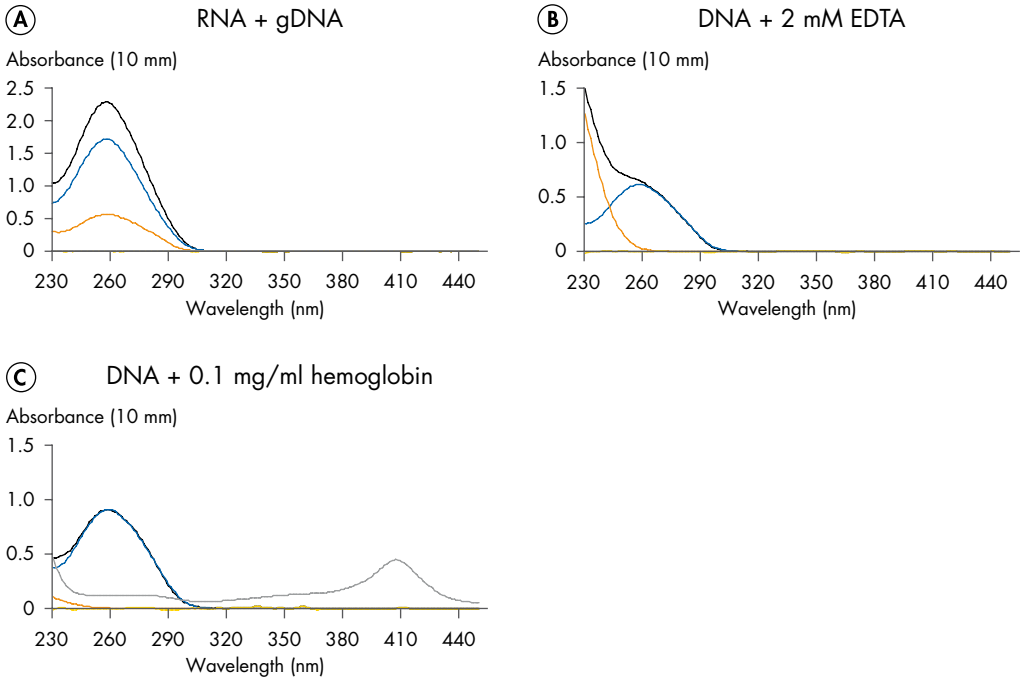

Figure	Sample	Total absorbance (A_{260})	Target concentration (ng/µl)	Measured concentration (ng/µl)	Impurities (A_{260})	Background (A_{260})
A	RNA + gDNA 30 ng/µl	2.22	70 (RNA)	69.1 (RNA)	0.50	0.00
B	DNA + 2 mM EDTA	0.89	45 (DNA)	42.7 (DNA)	0.04	0.00
C	DNA + 0.1 mg/ml hemoglobin	0.91	45 (DNA)	45.6 (DNA)	0.00	0.12

Figure 2. QIAxpert measurements with the RNA RNeasy App (Figure A) or with the DNA QIAsymphony App (Figures B and C). RNA and DNA concentrations and purity were assessed on the QIAxpert with different spiked-in contaminants: **A** gDNA, **B** EDTA and **C** hemoglobin. **Top** Spectral content profiling showing total measured spectrum (**black**), RNA/DNA spectrum (**blue**), impurities spectrum, including contaminants like DNA in A and EDTA in B (**orange**), residues (**yellow**; not visible, because no unknown components were detected) as well as background, including hemoglobin in C (**gray**). **Bottom** A table showing total absorbance values (A_{260}), target RNA/DNA concentrations (A_{260}), measured RNA/DNA concentrations (ng/µl), impurities values (A_{260}) and background values (A_{260}). These results show reliable RNA and DNA concentration measurements (blue curve and computed values) even in the presence of spiked-in contaminants (orange and gray curve and their corresponding computed values).

For spectral content profiling applications, each color corresponds to a specific spectral profile as indicated below:

- **White/Black**: Measured UV/Vis spectrum or raw spectrum of a sample. Represents total absorption spectra after subtraction of background signal.
- **Blue**: Profile of the molecule of interest extracted by the spectral profiling algorithms (depending on the chosen app or DNA or RNA, respectively).
- **Pink**: Shown as an alternative to the blue curve when spectral content profiling is not within specifications; all the nucleic acids (DNA, RNA and nucleotides) are grouped in this profile as total nucleic acids.
- **Orange**: Spectrum of all impurities detected (mainly chemicals from purification workflows included in the SCP apps; DNA contamination included in RNA app or vice versa).
- **Gray**: Background spectrum mostly arising from sample turbidity and impurities with absorbance in the Vis range of the spectrum, e.g., hemoglobin, chlorophyll, etc.
- **Yellow**: Residual spectrum, i.e., part of the measured spectrum that cannot be attributed to the reference profiles used in the SCP algorithm. The area under the curve of this residual spectrum relative to the area under the curve of the measured UV/Vis spectrum (white/black curve) gives the residue value in percentage as an indication of the quality of the spectral content profiling. High residue value (>2.5%) indicates the presence of high amount of unknown compounds in the sample.

Scope of the SCP analysis

The QIAxpert system can detect components absorbing in the recorded UV/Vis spectrum spanning from 230–750 nm with a measurement range of 0.03–40 OD (A_{260}, 10 mm path length equivalent; 1.5–2000 ng/µl dsDNA). Chemicals absorbing outside the UV/Vis spectrum or optically inactive chemicals do not contribute to the absorption spectra and are therefore not detected.

The reference spectra of RNA, DNA and known contaminants, such as salts and other absorbing chemicals often present in the purification chemistries, are unique and specific to the spectral content profiling app being used based on the application and the molecule of interest. Detected contaminants, i.e., contaminants for which the absorption spectra is included in the SCP app, will be reported under the "impurities" category. Other chemicals absorbing in the UV/Vis spectrum for which reference spectra is not included in the selected SCP app, will be treated as "residues" [6, 8, 9]. If the total absorption of the residues represents over 2.5% of the total absorbance of the sample, the SCP analysis is not performed as a reliable deconvolution of the measured spectrum is not ensured. A red cross instead of a green check on the result screen indicates the presence of unknown compounds in the sample and an unsuccessful SCP analysis (Figure 3).

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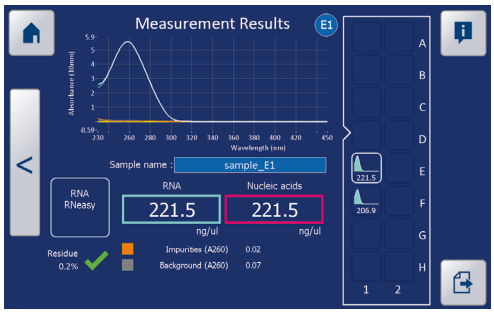

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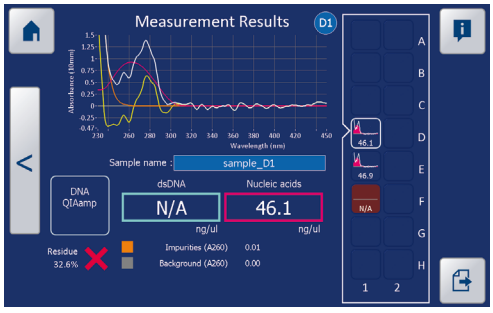

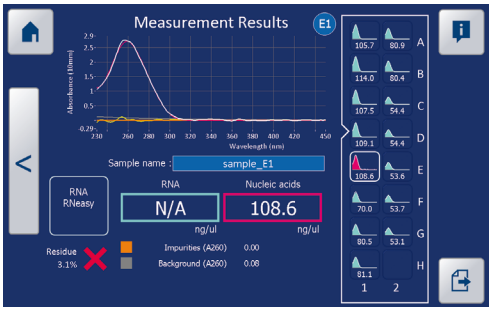

Figure 3. Screenshots of SCP results as seen on the QIAxpert screen. A RNA RNeasy SCP analysis of RNA sample. The success of the SCP analysis is indicated by a green check on the bottom left corner of the screen along with the percentage residue. **B** Failed SCP analyses with the DNA QIAamp app and the RNA RNeasy app due to sample contamination with residues (uncharacterized impurities) over 2.5%. A red cross indicates a failed SCP analysis; where DNA could not be discriminated from RNA with the DNA QIAamp app and vice versa with the RNA RNeasy app, and only the total nucleic acid concentrations are computed (pink curve). On the left side of the results screen, an overview of the slide and analyzed samples are provided. Light blue or pink histograms indicate the success or failure of the SCP analyses, respectively.

Although DNA and RNA have largely overlapping absorption spectra, spectral content profiling apps on the QIAxpert can perform spectral decomposition to selectively quantify DNA and RNA, where the concentration of total nucleic acids is ≥25 ng/µl with a 40–45% GC content. For concentration measurements falling below this threshold, spectral content profiling extracts the spectral contribution and computes the concentration of total nucleic acids (DNA, RNA and nucleotides), but still reports the profile of impurities present.

For spectral content profiling analyses, sample elution buffer as a blank is not required. The system provides automatic blanking for spectral content profiling corrections. Using sample storage buffer or solutions other than pure water (ddH_2O) as the blank can adversely affect the result [6, 7, 8, 9].

Conclusion

While classical spectrophotometry simply gives an overview of all absorbing components, computes concentration measurements and performs purity assessments solely based on the raw absorption spectrum, the QIAxpert system overcomes the limitations of conventional methods and offers the following unique benefits:

- Robust purity assessment. Impurities present in the samples can be detected and profiled. This insight into sample purity and composition lets users make informed decisions regarding sample use in downstream applications.
- Superior UV/Vis-based quantification. Accurate quantification of nucleic acids, not influenced by the presence of other UV/Vis-absorbing molecules that may interfere with the A_{260} or A_{280} absorbance readings.
- Dye-free discrimination of DNA from RNA. As these nucleic acids have slightly different absorption spectra, the QIAxpert can differentiate between these molecules and even detect gDNA contamination in RNA samples.

Spectral content profiling enables accurate and reliable quantification of the molecule of interest by subtracting other absorbing impurities and background turbidity from complex samples in a single measurement run. The result is a simple visual representation that reveals the true content of your samples, independent of buffer composition and level of contamination.

Discover more about the QIAxpert System through a **virtual demo**.

References

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