



IMAPlate™ 5RC96 Application Note

DNA/RNA quantification and quality analysis

Introduction

The quantification of DNA or RNA by the spectroscopy is a well-established routine method in many laboratories. The absorbance of UV light at wavelength of 260 nm by a pure DNA or RNA provides a simple and accurate estimation of the concentration of nucleic acids in a sample. For example, the absorption of 1 OD (A) is approximately equivalent to 50 µg/ml dsDNA, 33 µg/ml ssDNA, 40 µg/ml RNA or 30 µg/ml for oligonucleotides.

Besides, the quality of a DNA or RNA sample can also be checked by the spectroscopy. The ratio of A260/A280 or A260/A230 is used to estimate the contamination of the sample by protein or by substances such as carbohydrates, peptides, phenols or aromatic compounds, respectively. The pure DNA should have an A260/A280 ratio of approximately 1.8, whereas pure RNA should give a value of approximately 2.0. The ratio A260/A230 should be approximately 2.2 for pure samples.

IMAPlate™ 5RC96 is the world's first miniaturized analytical platform capable of manually performing high-throughput liquid transfer, analysis, reaction and assay. It comprises 96 identical, funnel-like reaction units positioned according to standard 96-well plate format and each reaction unit contains a 5 µl round reaction chamber with a light path of 5 mm long. The IMAPlate™ 5RC96 uses capillary force to confine samples in the bottomless reaction chambers and therefore the samples can spectroscopically be analyzed one-by-one in a microwell plate reader in any range of UV-VIS-IR spectra. The use of IMAPlate™ 5RC96 combined with a microwell plate reader is an ideal routing method for quantification and quality analysis of DNA or RNA samples, especially, with limited amount of sample volume.

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Experimental

Reagents and Materials

- DNA standards
- 96-well V-bottom plate
- Pipette
- IMAPlate™ 5RC96 and reader adaptor
- Microwell plate reader (with UV range)

Procedure A: to determine the concentration of samples with capillary force based liquid transfer (high-throughput)

1. Transfer 15 to 20 µl of a standard series of DNA solution to the assigned wells of a 96-well V bottom plate.
2. Transfer 15 to 20 µl of sample solutions to the rest well of the 96-well V bottom plate.
3. Aspirate the standard and sample solutions to IMAPlate™ 5RC96 by capillary force.
4. Place the IMAPlate™ 5RC96 in the reader with the adaptor.
5. Measure absorbance at wavelength of 260 nm and base line absorbance at wavelength of 350 nm.
6. Use true absorbance values ($A_{260} - A_{350}$) to calculate DNA concentration of samples according to the standards.

Procedure B: to determine the concentration of samples with pipette loading

1. Pipette 1, 2, 3, 4 and 5 µl of a standard DNA solution to the assigned reaction chambers on IMAPlate™ 5RC96.
2. Pipette appropriate amount of sample solutions (1 to 5 µl) to the rest reaction chambers on IMAPlate™ 5RC96 according to the expected concentration.
3. Place the IMAPlate™ 5RC96 in the reader with the adaptor.
4. Measure absorbance at wavelength of 260 nm and base line absorbance at wavelength of 350 nm.
5. Use true absorbance values ($A_{260} - A_{350}$) to calculate DNA concentration of samples according to the standards.



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Results and Discussion

Figure 1 shows spectra of calf thymus DNA solution and water in the reaction chambers of an IMAPlate™ 5RC96 measured by a microwell plate reader. The obtained spectrum is a typical spectrum of a pure DNA sample measured by a spectrophotometer and can be used for both quantitative and quality analysis.

The data of calf thymus DNA standards, generated with the **Procedure A**, can very well be fitted with linear regression equation (Figure 2). The coefficient of variation from 96 reaction chambers of an IMAPlate™ 5RC96 is 5.8 % for measuring a known concentration of calf thymus DNA solution in the low detection range (Figure 3). According to the results, the concentration of a DNA sample within 5 µg/ml to 250 µg/ml could accurately be determined by this procedure. The **Procedure A** is an easy, fast, robust and effective means for the analysis of a large number of samples.

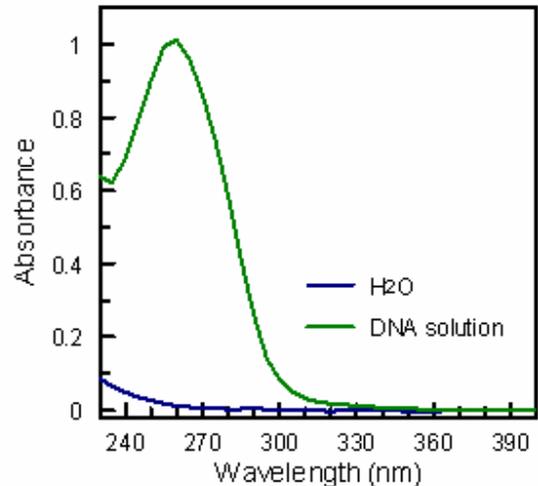


Fig 1. Spectra of pure DNA solution and water measured using an IMAPlate™ 5RC96

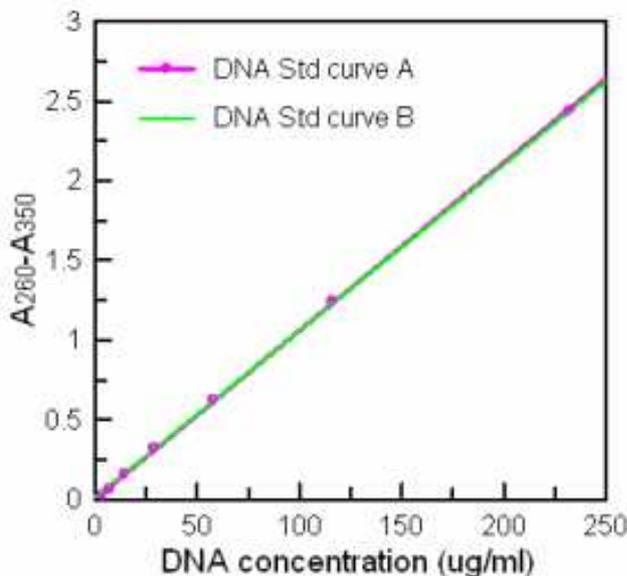


Fig 2. DNA standard curves generated from an IMAPlate™ 5RC96

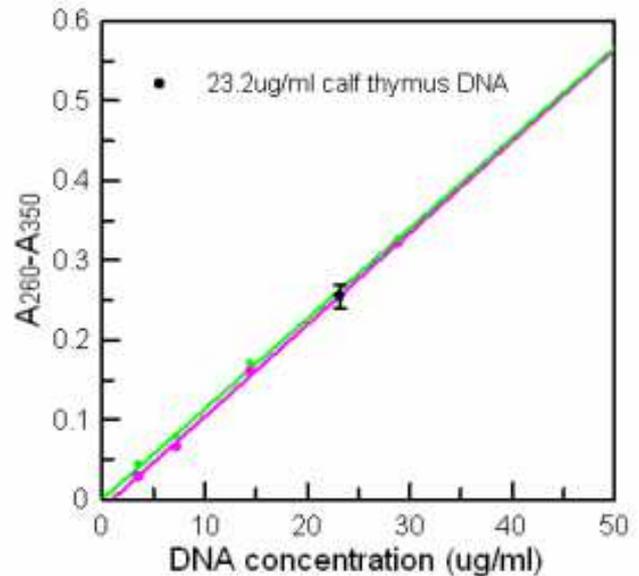


Fig 3. Distribution of 96 data from a known concentration sample on an IMAPlate™ 5RC96

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Figure 4 represents a diagram of the true absorbance against the volume of a DNA sample pipetted in the reaction chambers. The true absorbance shows a linearity relation with the volume at the range from 1 µl to 5 µl. Therefore, the use of a pipette can provide an alternative to prepare a standard curve, in which only one standard with an appropriate concentration can be added into the assigned reaction chambers on IMAPlate™ 5RC96 with a serial volume. In such a way, the preparation of a standard series of DNA solution is no longer necessary. It may also eliminate the need for dilution of samples with high concentration just by adding 1 µl of samples to the reaction chambers for measurement.

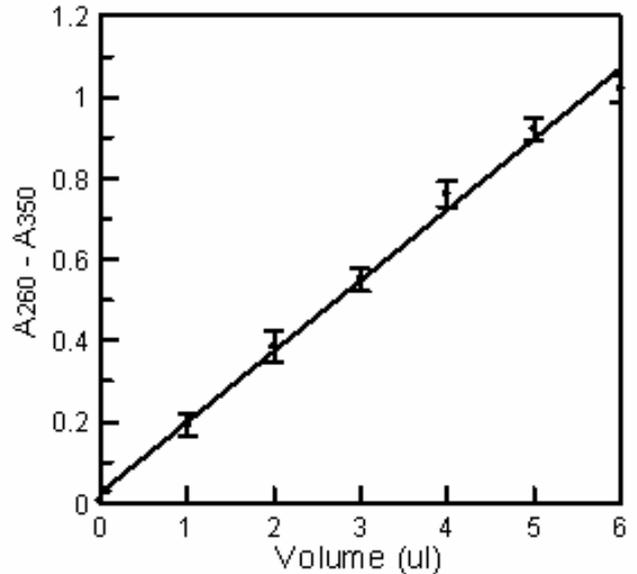


Fig 4. Relationship between the true absorbance and the volume of DNA solution added in the reaction chamber

Conclusion

The IMAPlate™ 5RC96 is an easy-to-use, robust, miniaturized analytical platform suitable for quantification and quality analysis of samples with 96-well plate readers. It offers users a wide range of UV-VIS-IR spectrometric analysis of 1 µl to 5 µl of samples and a variable light path from 1 mm to 5 mm. The measured samples can totally be recovered without cross contamination risk. The flexible, simple handling of the IMAPlate™ 5RC96 not only saves hands on time and material cost for the analysis, but also enhances the lab productivity.

Products information

Catalog No:	Article	Contents	Quantity
NCL-STK-001	IMAPlate™ 5RC96 Start Kit	IMAPlate™ 5RC96 Reader adaptor (adjustable) Swiss army knife (tool for adjusting reader adaptor)	5 ea 1 ea 1 ea
NCL-P5W-002	IMAPlate™ 5RC96	IMAPlate™ 5RC96	5 ea
NCL-P25W-003	IMAPlate™ 5RC96	IMAPlate™ 5RC96 Swiss army knife	25 ea 1 ea

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