Supelco®
Analytical Products

Application Note

Determination of ssDNA in purified solutions

UV-spectrophotometric direct measurement at 260 nm

Introduction

The quantification of DNA in solutions is an important application in bioanalytics.

In purified DNA solutions, for example, their assay can be relatively easily and swiftly determined by spectrophotometric measurement of the optical density (absorption) at the 260 nm wavelength.

At this wavelength, DNA – both, single-stranded (ssDNA) as well as double-stranded (dsDNA) - and RNA exhibit their absorption maximum. Depending on the DNA concentration, the absorption/optical density increases linearly in an absorption range from 0.1-1.5 Abs. Following the Beer-Lambert law, specific factors can be used to draw conclusions regarding the concentration of ssDNA, dsDNA, and RNA in the sample. These factors are calculated from the respective specific absorption coefficients. At an OD_{260} value of 1 and an optical path length of 10 mm, the following factors are calculated:

- ssDNA 33 µg/mL
- dsDNA 50 µg/mL
- ssRNA 40 µg/mL

DNA purity

In many cases, DNA solutions are contaminated with proteins or organic compounds that have been used to purify the DNA.

One advantage of the spectrophotometric method is that - in addition to quantifying the DNA content - it also allows statements about the purity of the DNA.

Proteins, for example, absorb light at a wavelength of 280 nm. If the solution contains proteins, the optical density at the 280 nm wavelength rises. The ratio of the absorption values of 260 nm to 280 nm can be used to estimate the purity of the solution.

In pure DNA solutions, the reference value for the OD_{260}/OD_{280} ratio is 1.8, for pure RNA solutions the reference value is 2.0. When the measured value is lower, it can be assumed that proteins are present in the solution.

Besides the OD_{260}/OD_{280} ratio, the OD_{260}/OD_{230} ratio is also frequently calculated. At the 230 nm wavelength, organic compounds absorb such as carbohydrates, guanidine and phenol, which is frequently used as an extraction agent. Their absorption at 230 nm produces a reduction in the OD_{260}/OD_{230} ratio. As with the OD_{260}/OD_{280} ratio, a reference value of 1.8 applies for DNA solutions and one of 2.0 for RNA solutions [1].

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Experimental

This Application Note describes the quantification of ssDNA in samples. The analysis is quick and easy. The method is preprogrammed on the corresponding Spectroquant® Prove 300 and Spectroquant® Prove 600 photometers with firmware version 1.5 or above.

Separate application notes are available for dsDNA and RNA.

Method

The optical density OD (absorption) is measured at 230, 260 and 280 nm. The ssDNA concentration is calculated on basis of the OD_{260} value. The ratios OD_{260}/OD_{280} and OD_{260}/OD_{230} allow statements about the purity of the DNA-solution.

Measuring range

3–25000 µg/mL ssDNA (method no. 2511)

Sample material

Purified ssDNA solutions

Reagents, Instruments and Materials:

Instruments

For the ssDNA measurement one of the following Spectroquant® photometers is necessary:

Cat. No.	Product
1.73028	Spectroquant® UV/VIS Spectrophotometer Prove 600 plus
1.73027	Spectroquant® UV/VIS Spectrophotometer Prove 300 plus

Also first generation Prove instruments are compatible and preprogrammed with this method.

Materials

Cat. No.	Product
100784	Rectangular quartz cell 10 mm or
Z600288	Semi-micro rectangular quartz cell 10 mm*

*Due to the optical characteristics of the Prove Spectrophotometers the use of 10 mm micro cells is not possible. Plus, due to the automatic cell detection of the Prove instruments it is important to use semi-micro cells with complete side walls

Analytical approach

Sample preparation

- Homogenize samples by swirling carefully
- Dilute the sample, if necessary. For ssDNA concentrations > 45 µg/mL a dilution is necessary. The following table gives suggestions how to dilute the sample depending on the estimated ssDNA concentration. Please note that the **minimum sample volume is 0.6 mL for the 10 mm semi-micro cell or 1.5 mL for the 10 mm cell.** The maximal possible sample dilution factor is 500 (dilution 1+499).
- It is recommended to dilute with the buffer used as sample solvent. The optimal buffer has a pH value of 7.5–8.5 and a low salt content, e.g. TE-buffer. The usage of buffers ensures a higher reproducibility in comparison to water due to a stable pH value, while a low salt content ensures fewer interferences during measurement. [1, 2].

Materials

Estimated ssDNA concentration [µg/mL]	Dilution	Exemplary dilution procedure
3-4	-	Use the sample undiluted
45-900	1+19	Pipette 0.250 mL sample into test tube and add 4.75 mL solvent.
900-4500	1+99	Pipette 0.050 mL sample into test tube and add 4.95 mL solvent
4500-25000	1+499	Pipette 0.010 mL sample into test tube and add 4.99 mL solvent

Note

Depending on the DNA concentration, the absorption/ optical density increases linearly in an absorption range from 0.1-1.5 Abs at 260 nm. The instrument checks automatically if the measured absorbance of the sample is in this range. If the absorbance is higher than 1.5 Abs a message appears **"Condition not met – higher dilution necessary".** If the absorbance is below 0.1 Abs, no result will be displayed, the instrument shows "---" instead of a result

Preparing the measurement solutions

Blank for zero adjustment

For zero adjustment use the sample solvent (e.g. used buffer).

Measurement sample

For the measurement sample use the prepared sample solution.

Measurement

- Open the method list (<Methods>) and select method No. 2511 “ssDNA”.
- For each measurement series, a zero adjustment is required. It is recommended to use the same cell for zero adjustment and for sample measurement. The zeroing procedure for the measurement series is automatically prompted by the instrument. For zero adjustment fill the 10-mm rectangular quartz cell with the solvent used for dilution. After prompting, place the filled rectangular quartz cell in the cell compartment, the zero adjustment is executed automatically. Confirm the implementation of zero adjustment with **"OK"**. The zero adjustment is valid for the entire measurement series

- The sample dilution must be entered. An input mask pops up. Enter the dilution in the form 1+x and tap **<OK>** to confirm.
- Fill the measurement sample into the same or a matched 10-mm rectangular quartz cell and insert the cell into the cell compartment. The measurement starts automatically.
- Tap **<OK>** to confirm.
- Read off the results from the display.

Results

The following results are given by the instrument:

- ssDNA concentration in µg/mL
- OD_{260}/OD_{280} ratio
- OD_{260}/OD_{230} ratio
- Optical densities (absorptions) at 230, 260 and 280 nm

Data transfer Prove spectrophotometers

After measurement transfer the values measured on the Prove spectrophotometer using Prove Connect to LIMS.

Adjustments

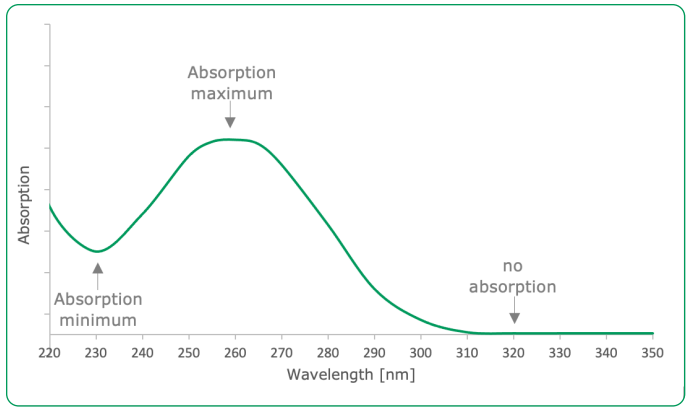
The preprogrammed ssDNA factor of 33 may differ under given circumstances from the actual factor. This is because the factor is dependent on the content of the nucleic bases and correspondingly may differ from one DNA sample to the next. For the case that the exact ssDNA factor is known and the objective is to enhance the accuracy of the measurement, it is possible to adapt the ssDNA factor in the method: Select method 2511, close the input mask by pressing “X”. Press "Settings" and select "Factors" from the list.

Tap on the entry field "ssDNA Factor", enter the corrected ssDNA factor, and confirm with **"OK".**

Interferences

As described above proteins or organic substances can interfere with the measurement. By measuring the OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios it can be checked whether any such substances are present in the sample. In case of doubt run a spectrum from 230–320 nm. Pure DNA solutions should have an absorption maximum at 260 nm and an absorption minimum at ca. 230 nm. Moreover, the spectrum should show no absorption at 320 nm.

Another substance that interferes the analysis is RNA, which absorption spectrum is like that of DNA.



Conclusion

The measurement of the optical density at 260 nm is a fast and easy way to quantify the ssDNA content of your sample. By measuring the OD260/OD280 and OD260/OD230 ratios it can be checked if the sample is contaminated with proteins and other organic compounds.

The method is preprogrammed in the Spectroquant® Prove 300 and 600, so there is no need for a manual calculation of the results. The results can be read of directly from the instrument display.

For more information

Spectroquant® Photometry System see:
SigmaAldrich.com/photometry

References

1. R. E. Farell, RNA Methodologies: Laboratory Guide for Isolation and Characterization, 5. Edition, Elsevier, 2017.
2. Oxford Gene Technology, Understanding and measuring variations in DNA sample quality, 08/2011.

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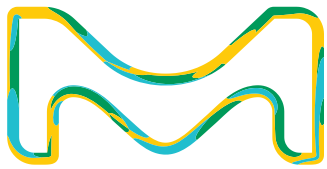

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01/2024