Supelco®
Analytical Products

Application Note

# Determination of RNA in Purified Solutions

## UV-spectrophotometric direct measurement at 260 nm

### Introduction

The quantification of RNA in solutions is an important application in bioanalytics. In purified RNA 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, RNA solutions are contaminated with proteins or organic compounds that have been used to purify the RNA.

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

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].


### Experimental

This Application Note describes the quantification of RNA 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 ssDNA and dsDNA.

#### Method

The optical density OD (absorption) is measured at 230, 260 and 280 nm. The RNA 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 RNA-solution.

#### Measuring range

4–30000 µg/ml RNA (method no. 2510)

#### Sample material

Purified RNA solutions

## Reagents, Instruments and Materials:

| Cat. No. | Description |
|---|---|
| **Instruments** | |
| For the RNA measurement one of the following Spectroquant® photometers is necessary: | |
| **1.73027** | Spectroquant® UV/VIS Spectrophotometer Prove 300 plus |
| **1.73028** | Spectroquant® UV/VIS Spectrophotometer Prove 600 plus |
| **Software for data maintenance** | |
| The Spectroquant® Prove Connect to LIMS software package provides an easy way to transfer your data into a preexisting LIMS system. This software can be purchased under: | |
| **Y11086** | Prove Connect to LIMS |
| **Materials** | |
| **100784** | Rectangular quartz cell 10 mm or |
| **Z600288** | Semi-micro rectangular quartz cell 10 mm* Pipettes |

Also first generation Prove instruments are compatible and preprogrammed with this method.
* 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 RNA concentrations > 60 µg/ml a dilution is necessary. The following table gives suggestions how to dilute the sample depending on the estimated RNA 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]

| Estimated RNA concentration [µg/ml]t | Dilution | Exemplary dilution procedure |
|---|---|---|
| 4–60 | - | Use the sample undiluted |
| 60-1200 | 1+19 | Pipette 0.250 ml sample into test tube and add 4.75 ml solvent. |
| 1200-6000 | 1+99 | Pipette 0.050 ml sample into test tube and add 4.95 ml solvent. |
| 6000–30000 | 1+499 | Pipette 0.010 ml sample into test tube and add 4.99 ml solvent. |

### Note

Depending on the RNA 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. 2510 "RNA".
- 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:

- RNA 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 (optional)

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

## Adjustment:

The preprogrammed RNA factor of 40 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 RNA sample to the next. For the case that the exact RNA factor is known and the objective is to enhance the accuracy of the measurement, it is possible to adapt the RNA factor in the method:

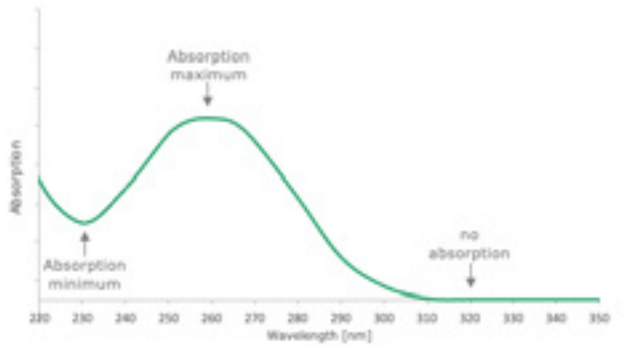
Select method 2510, close the input mask by pressing “X”. Press "Settings" and select "Factors" from the list.

Tap on the entry field "RNA Factor", enter the corrected RNA 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 RNA 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 DNA, which absorption spectrum is like that of RNA.




## Conclusion

The measurement of the optical density at 260 nm is a fast and easy way to quantify the RNA content of your sample. By measuring the $OD_{260}/OD_{280}$ and $OD_{260}/OD_{230}$ 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
**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.

Link: https://www.ogt.com/resources/literature/483_understanding_and_measuring_variations_in_dna_sample_quality

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