

Colibri Microvolume Spectrometer

Low volume measurement of RNA

Introduction

Measurements of nucleic acids in microvolumes have become a routine operation in many life science laboratories. As the nitrogenous bases in nucleotides absorb light at 260 nm, this wavelength can be used to determine RNA concentration. Typical applications are quantification of RNA after isolation, purity or integrity of RNA.

Extinction coefficients of nucleic acids are well established:

Based on the average extinction at 1 OD of $0.020 (\mu\text{g/ml})^{-1} \text{cm}^{-1}$ for double-stranded DNA, $0.027 (\mu\text{g/ml})^{-1} \text{cm}^{-1}$ for single stranded DNA and $0.025 (\mu\text{g/ml})^{-1} \text{cm}^{-1}$ for RNA, nucleic acid measurements can be done without using a standard curve.

Materials

1. Colibri Microvolume Spectrometer by Titertek-Berthold
2. RNA (Babc 28.9)
3. ddH₂O, pipette and tips, microfuge tubes

Methods

Sample concentration will be automatically calculated by Colibri. Different protocols are available for each nucleic acid type.

Nucleic Acids	Extinction coefficient [$\text{cm}^{-1} \text{M}^{-1}$]	Colibri protocol
RNA	40	RNA-40
Single stranded DNA	33	ssDNA-33
Double stranded DNA	50	dsDNA-50

Here we report the measurement of RNA.

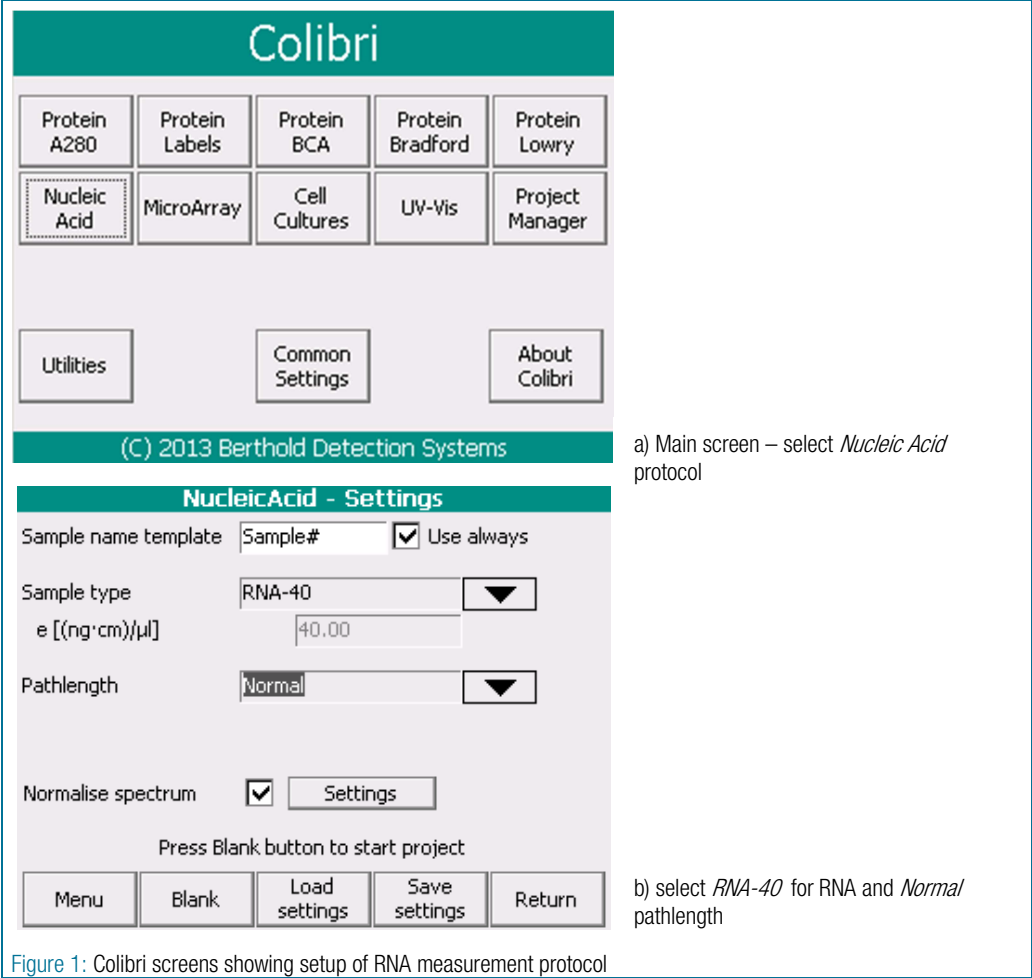
In a first step the concentration of Babc 28.9 was determined. 2 μl were measured in triplicates. Afterwards the stock solution was diluted 1:5 with ddH₂O. A 1:2 dilution series in ddH₂O was prepared. Measurements were taken in triplicates, measurement volume was 2 μl each. Prior to each measurement series the blank was determined by using ddH₂O.

Software settings:

Protocol: Nucleic Acid, RNA-40

Pathlength: Normal (1mm)

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a) Main screen – select *Nucleic Acid* protocol

b) select *RNA-40* for RNA and *Normal* pathlength

Figure 1: Colibri screens showing setup of RNA measurement protocol

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Results:

1. Concentration

Concentration of the Babc 28.9 stock solution was calculated 737.68 ng/μl.

1. Dilution series

Concentration [ng/μl]	Measurement values 260 nm [OD]			Mean	Standard Deviation	CV [%]
147.54	4.542	4.539	4.552	4.544	0.005	0.12%
73.77	2.398	2.401	2.411	2.403	0.005	0.23%
36.88	1.114	1.117	1.111	1.114	0.003	0.23%
18.44	0.525	0.530	0.538	0.531	0.005	0.97%
9.22	0.300	0.303	0.305	0.303	0.002	0.70%
4.61	0.206	0.204	0.206	0.205	0.001	0.32%
2.31	0.136	0.137	0.143	0.139	0.003	1.98%

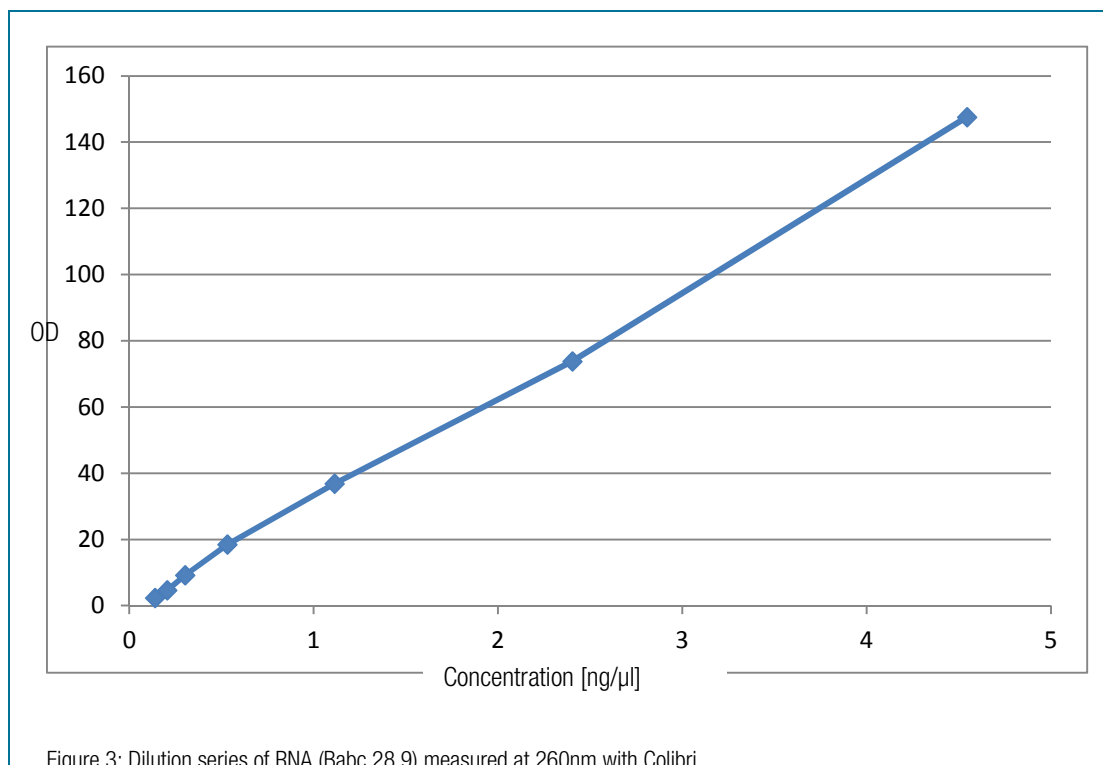


Figure 3: Dilution series of RNA (Babc 28.9) measured at 260nm with Colibri

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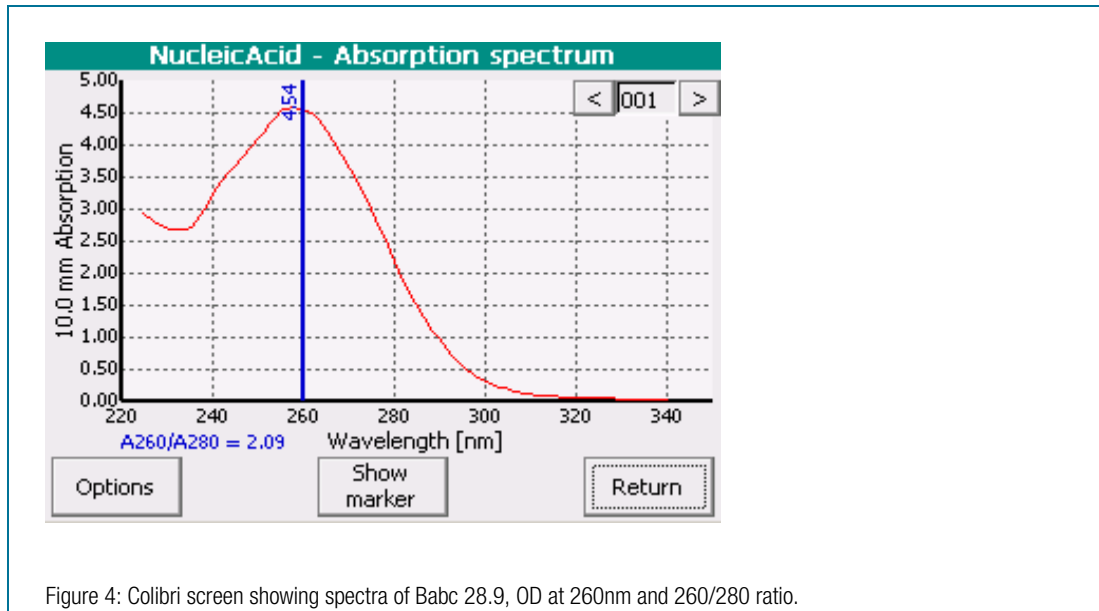


Figure 4: Colibri screen showing spectra of Babc 28.9, OD at 260nm and 260/280 ratio.

Conclusion:

The Colibri microvolume spectrometer shows excellent linearity and sensitivity down to 1ng/μL RNA. Predefined protocols and automatic calculation of sample concentration makes measurement fast and convenient.

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