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Berthold Detection Systems GmbH
Bleichstrasse 56–68
D-75173 Pforzheim/Germany

Phone: +49(0)7231/9206-0
Fax: +49(0)7231/9206-50
E-Mail: contact@titertek-berthold.com
Internet: www.titertek-berthold.com

Colibri Microvolume Spectrometer

Low volume measurement of ssDNA

Introduction

Measurements of DNA in microvolumes have become a routine operation in many life science laboratories. As the nitrogeneous bases in nucleotides absorb light at 260 nm, this wavelength can be used to determine the DNA concentration. Typical applications are quantification of template prior to sequencing and PCR or measurement of sample purity.

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

Materials

1. Colibri Microvolume Spectrometer by Titertek-Berthold
2. Oligonucleotide (26 bases, X11 ClaI for 5'-cgatcgatatgaaaaaggacttttg cttacacgg-3', purchased at biomers.net)
3. ddH_2O, pipette and tips, microfuge tubes

Methods

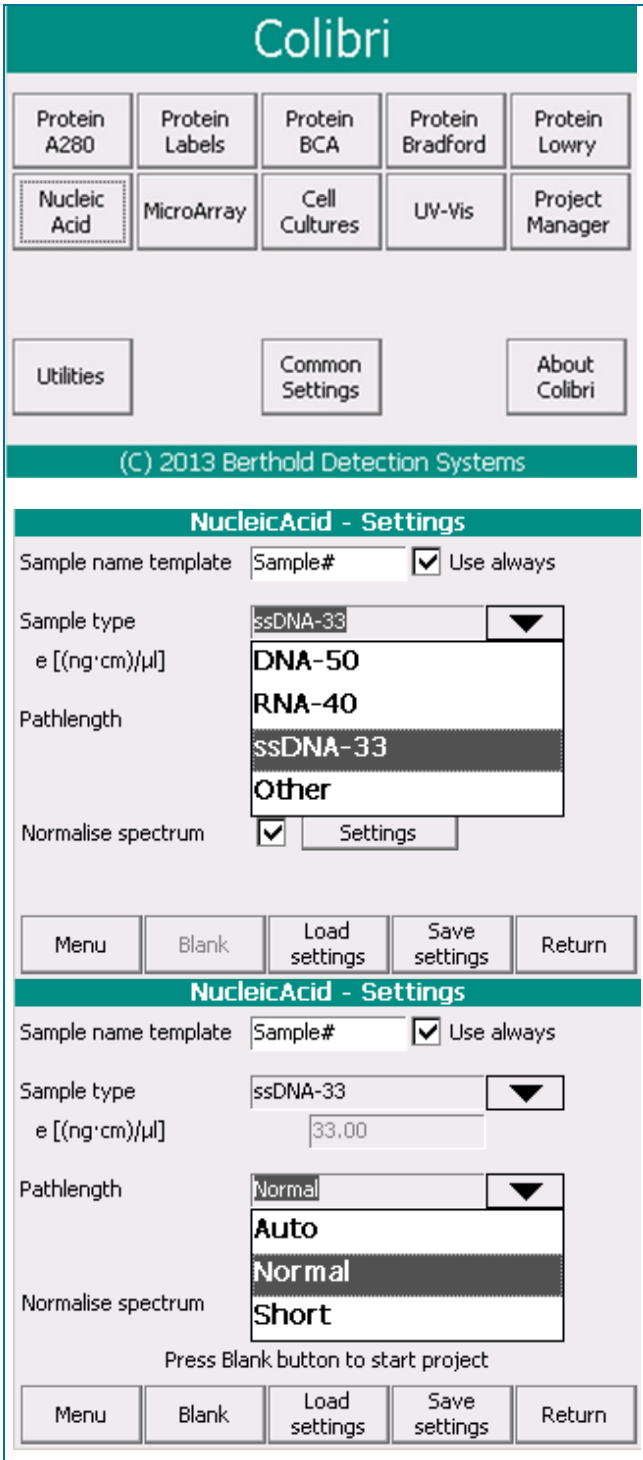
Sample concentration will be automatically calculated by Colibri. Different protocols are available for each nucleic acid type.

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

Here we report the measurement of ssDNA Oligonucleotide.
In a first step the concentration of the oligonucleotide stock solution was determined. 2 µl were measured in quadruplicates. Afterwards the stock solution was diluted 1:5 with ddH_2O. A 1:2 dilution series in ddH_2O was prepared. Measurements were taken in triplicates, measurement volume was 2 µl each.
Prior to each measurement series the blank was determined by using ddH_2O.

Software settings:

Protocol: Nucleic Acid, ssDNA-33
Pathlength: Normal (1mm)



a) Main screen – select *Nucleic Acid* protocol

b) select *ssDNA-33* for single stranded DNA

c) select *Normal* pathlength

Figure 1: Colibri screens showing setup of a ssDNA measurement protocol

Results:

1. Concentration

Concentration of the oligonucleotide stock solution was calculated 647.75 ng/µl.

2. Dilution series

Dilution (stock solution)	Measurement values 260nm [OD]			Mean	Standard Deviation	CV of meas. values [%]
1:5	6,094	6,087	6,078	6,086	0,0065	0.11%
1:10	2,877	2,882	2,888	2,882	0,0045	0.16%
1:20	1,307	1,309	1,318	1,311	0,0048	0.36%
1:40	0,591	0,589	0,595	0,592	0,0025	0.42%
1:80	0,254	0,258	0,259	0,257	0,0022	0.84%
1:160	0,101	0,105	0,112	0,106	0,0045	4.29%
1:320	0,033	0,036	0,036	0,035	0,0014	4.04%

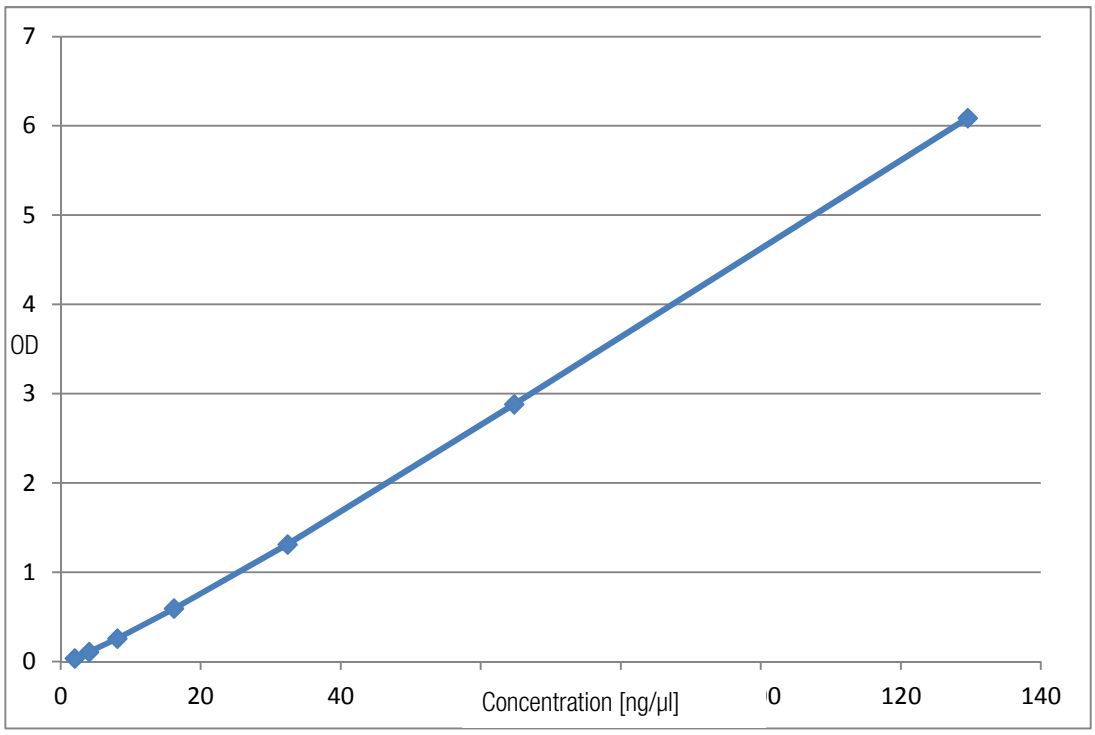


Figure 3. Dilution series of ssDNA oligonucleotide measured at 260nm with Colibri

Conclusion:

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

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