

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 Clal for 5'-cgatcgatatgaaaaaggacttttg cttacacgg-3', purchased at biomers.net)
- 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 [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 μ l were measured in quadruplicates. 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 μ l each. Prior to each measurement series the blank was determined by using ddH₂O.

Software settings:

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

Colibri Microvolume Spectrometer



		Colibr	i		
Protein A280	Protein Labels	Protein BCA	Protein Bradford	Protein Lowry	
Nucleic Acid	MicroArray	Cell Cultures	UV-Vis	Project Manager	a) Main annan - aclast <i>Musicia</i> da
	_				a) Main screen – select <i>Nucleic Ach</i> protocol
Utilities		Common Settings		About Colibri	
(rthold Deteo		ns	
Sample nam		sicAcid - Se Sample#	ettings Use al	wavs	
Sample type		ssDNA-33		—	
e [(ng·cm)	÷	DNA-50		•	
		RNA-40			b) poloot apply 22 for single stress
Pathlength		b) select <i>ssDNA-33</i> for single strand DNA			
		Other			
Normalise s	pectrum	Setti	ings		
Menu	Blank	Load settings	Save settings	Return	
	Nucle	eicAcid - Se			
Sample nam		Sample#	🔽 Use al	lways	
Sample type	e [ssDNA-33			
e [(ng·cm)		33.00		<u> </u>	
Pathlength	[Normal			
gar	ż	Auto			
		Normal			
Normalise s	pectrum	Short			
Press Blank button to start project					a) coloct //
Menu	Blank	Load settings	Save settings	Return	c) select <i>Normal</i> pathlength
Figure 1: Colil	bri screens sho			urement protocol	

Colibri Microvolume Spectrometer



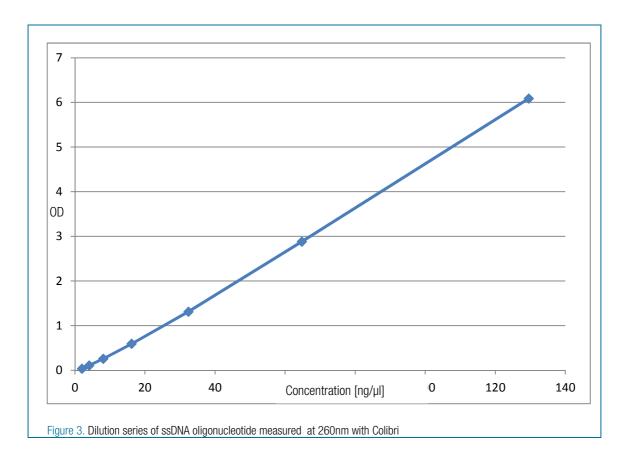
Results:

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%



Colibri Microvolume Spectrometer



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.

Acknowledgement:

Samples and labspace provided by Sarah Becker, Department of Cell and Developmental Biology, KIT Karlsruhe, Germany,