

Spectrophotometric Quantification of Nucleic Acids

LabelGuard™ enables photometric quantification of submicroliter samples using a standard photometer

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Introduction

The analysis of nucleic acids is part of the daily routine in molecular biology labs. DNA and RNA concentrations, the purity, and in case of modified nucleic acids, the calculation of modification rates mostly are determined utilizing UV/Vis spectroscopy.

In standard UV/Vis spectrophotometers generally cuvettes with a pathlength of 1 cm are used. This setup has main limitations for nucleic acid measurements. Sample volumes of 50 to 1000 µl are required in most cases with the need of sample dilution in order to stay in the linear range of the spectrophotometer.

Depending on the application in most cases the mainly precious sample is not reusable after the measurement.

The LabelGuard™ Microliter Cell handles these limitations in an optimized manner. By choosing one of two lids with pathlengths of 1 mm or 0.2 mm a virtual dilution factor of 1:10 or 1:50 without the need of physically diluting the sample is achieved. In addition the volumes needed for the measurements



are with 3 µl for the 1 mm lid and even less than 1 µl for the 0.2 mm lid very low. The high reproducibility, avoidance of dilution errors, and the possibility to retrieve the samples after the measurement, are the outstanding features of the LabelGuard™ Microliter Cell. In addition using the LabelGuard™ Microliter Cell time and money consumption in comparison to quartz glass cuvettes will be minimized. For these reasons the LabelGuard™ Microliter Cell is a very useful analysis tool for technologies like Real-Time PCR, Laser Capture Microdissection und Microarray based applications. Besides these nucleic acid applications the LabelGuard™ Microliter Cell is also suitable for protein quantification like measurement of the absorbency at 280 nm, BCA, Bradford and Lowry.

Technical specifications

The innovative optical pathway of the cell is designed for optimum measurement results with very low sample volumes (Figure 1).

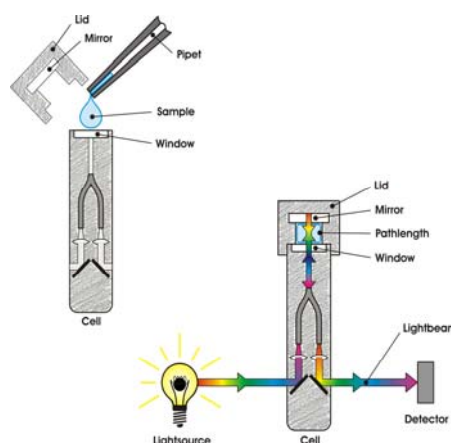


Figure 1: Principle of measurement

The sample is pipetted directly onto the centre of the measuring window. Due to the integrated beam deflection and the use of fibre-optic light conductors the sample can be measured directly on the measurement window. Using the 1 mm or 0.2 mm lid creates a liquid column of defined pathlength of 1 mm and 0.2 mm, respectively. This generates virtual dilution factors of 1:10 or 1:50 in comparison to a standard 1 cm cuvette measurement, which saves time and avoids dilution errors. The required sample volume for the 1 mm lid is 3 µl to 5 µl and for the 0.2 mm lid 0.7 µl to 4 µl.

The mean dynamic range depends strongly on the type of the photometer used. With the LabelGuard™ Microliter cell the average dynamic range for ds-DNA is between 2 ng/µl and 5.000 ng/µl. The dimensions of the LabelGuard™ Microliter Cell with a footprint of 12.5 mm to 12.5 mm are similar to standard cuvettes. The centre height of the measuring window is adjustable with adapters to the most common used centre heights. Therefore the cell is compatible with most commercially available spectrophotometers. The available wavelength range is 190 nm to 800 nm (Figure 2).

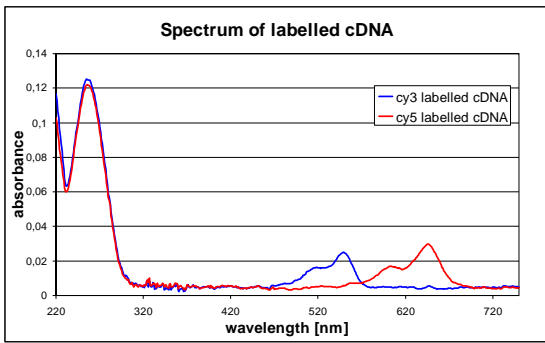
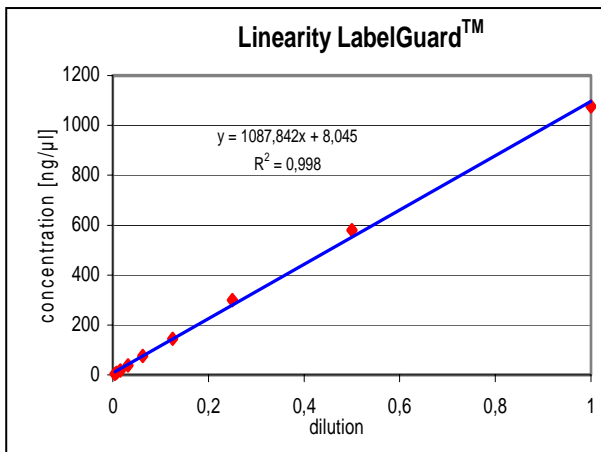


Figure 2: Spectrum of labelled cDNA
 Samples of cyanine 3 and cyanine 5 labelled cDNA were measured over a wavelength range from 220 nm to 750 nm with the SPECORD 50 from Analytik Jena.

Experimental specifications

Different approaches were selected to validate the performance of the LabelGuard™ Microliter Cell.

Measuring the absorbance of ds-DNA at 260 nm over a broad concentration range with three different spectrophotometers shows a very high linearity (Figure 3, Table 1).



Dilution	Concentration [ng/µl]
undiluted	1076,83
1/2	580,50
1/4	300,33
1/8	145,17
1/16	75,17
1/32	37,17
1/64	16,67
1/128	8,50
1/256	3,50

Figure 3, Table 1: Linearity
 The linearity has been determined with the spectrophotometer SPECORD 210 from Analytik Jena. A dilution series of ds-DNA has been measured in triplicates. The resulting data show a very good linearity ($R^2=0.998$) over a broad range of concentrations. Similar experiments performed with the SPEKOL 1300 photometer from Analytik Jena and with the BioPhotometer from Eppendorf confirm this high linearity.

The reproducibility of the LabelGuard™ Microliter Cell has been tested by measuring the concentrations of plasmid DNA and oligonucleotide probes on an Eppendorf BioPhotometer at 260 nm. Both available lids and different sample volumes were used (Table 2, Table 3). With coefficients of variation (c.v.) between 1.2% and 3.1% the reproducibility of the measured concentrations has been excellent.

No.	1 mm lid		0.2 mm lid	
	A260	µg/ml	A260	µg/ml
1	0,056	28,0	0,074	185,0
2	0,056	28,0	0,075	187,5
3	0,055	27,5	0,075	187,5
4	0,055	27,5	0,077	192,5
5	0,056	28,0	0,075	187,5
6	0,053	26,5	0,074	185,0
7	0,054	27,0	0,075	187,5
8	0,053	26,5	0,076	190,0
9	0,052	26,0	0,075	187,5
10	0,055	27,5	0,075	187,5
11	0,053	26,5	0,075	187,5
12	0,055	27,5	0,076	190,0
13	0,054	27,0	0,077	192,5
14	0,054	27,0	0,076	190,0
MW	0,054	27,2	0,075	188,4
StDev	0,001	0,6	0,001	2,3
c.v. %	1,9	2,2	1,3	1,2

Table 2: Reproducibility with plasmid DNA
 14 independent measurements with the 1 mm and the 0.2 mm lid were carried out with 3 and 1 µl of sample volume in an Eppendorf BioPhotometer. With an absorbance of 0.05 and 0.07 a concentration range at the lower detection limit of the spectrophotometer has been selected.

No.	µg/µl oligo	A260	volume
1	0,570	0,380	3 µl
2	0,576	0,384	2 µl
3	0,572	0,381	1 µl
4	0,585	0,390	1 µl
5	0,567	0,378	1 µl
6	0,620	0,413	1 µl
7	0,569	0,379	700 nl
8	0,570	0,380	700 nl
MW	0,578	0,386	
StDev	0,018	0,012	
c.v. %	3,1	3,1	

Table 3: Reproducibility with oligonucleotide probes
 8 independent measurements with a DNA oligonucleotide probe (20mer, Metabion) were performed with a sample volume of 700 nl to 3 µl using the 0.2 mm lid in an Eppendorf BioPhotometer. Despite the broad range of different sample volumes the coefficient of variation with 3.1% is extremely convincing.

As there are no disposables used with the LabelGuard™ Microliter Cell the cleaning of the measurement window is important. Due to the good accessibility of all parts of the cell the cleaning protocol is very straightforward. After the measurement the LabelGuard™ Microliter Cell remains in the cuvette holder. The lid is taken off and the sample can be retrieved and stored for later use. The measurement window and the lid should be cleaned with a fluff free swab or Kimwipe. If necessary residual fluffs should be removed completely from the measurement window and the lid with dry compressed air to ensure optimal performance. Alternate measurements of DNA and buffer at 260 nm show that possible systematic carryover effects can be excluded (Figure 4).

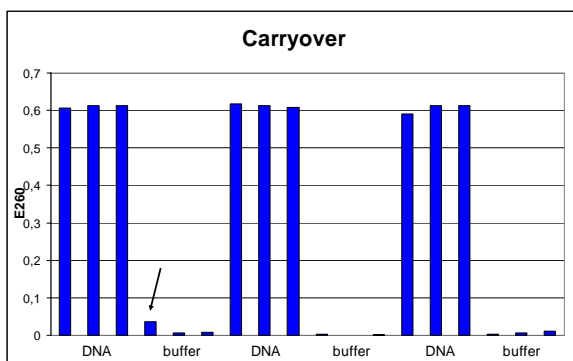


Figure 4: Carryover effects
Three measurements with high concentrations followed by three measurements with buffer were performed with the Eppendorf BioPhotometer. It could be clearly proved that there are no systematic carryover effects. Nevertheless, a minimal sample carryover (6%) at the fourth measurement (arrow) has been observed, demonstrating the importance of a careful cleaning of the microliter cell.

RNA measurements performed with the Eppendorf BioPhotometer at 260 nm with a standard quartz cuvette correlate with measurements performed with the LabelGuard™ Microliter Cell (Figure 5). Even though the concentration range has been selected in a very sensitive region the values are absolutely comparable.

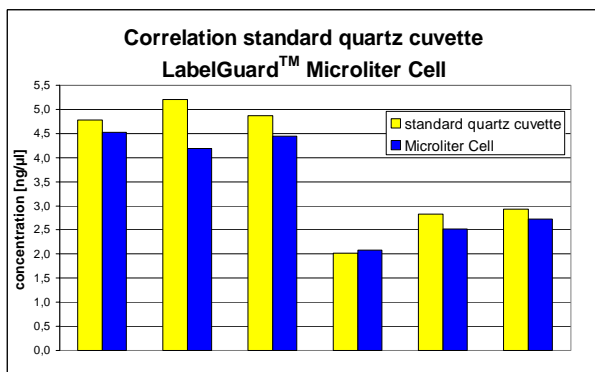


Figure 5: Quartz cuvette and LabelGuard™
6 different RNA samples were measured with both systems in triplicates and the recorded mean values are shown.

To compare the compatibility of the LabelGuard™ Microliter Cell with different spectrophotometers the identical DNA sample was measured in triplicates with the BioPhotometer from Eppendorf and the SPECORD 50 from Analytik Jena. The obtained concentration values are absolutely comparable (Figure 6).

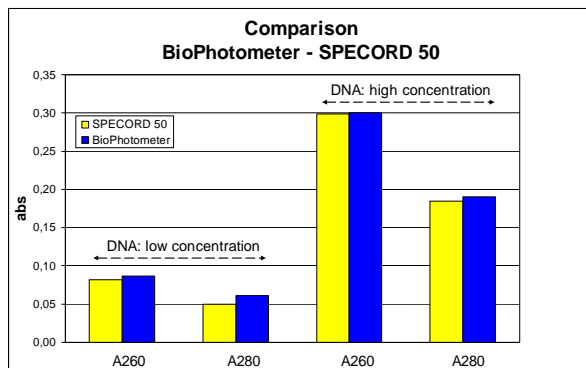


Figure 6: Comparison of different photometers
Low DNA concentrations were measured with the 0.2 mm lid, high concentrations with the 1 mm lid in triplicates. The resulting absorbance values of the low as well as of the high concentration measurements at 260 nm and 280 nm are very consistent and independent from the spectrophotometer used.

Conclusion

The LabelGuard™ Microliter Cell enables absolute reliable spectrophotometrical analysis of ultra low volume nucleic acid and protein samples. The LabelGuard™ Microliter Cell can be used in most commercial spectrophotometers with sample volumes starting from less than 1 μl. Avoiding the mostly necessary dilution of samples, possible error sources can be eliminated in comparison to standard glass or plastic cuvette measurements. The simple handling and cleaning procedure of the LabelGuard™ Microliter Cell saves hands on time as well as material cost for the spectrophotometrical analysis.

The LabelGuard™ Microliter Cell is a powerful analysis tool for technologies like Real-Time PCR, Laser Capture Microdissection und Microarray based applications.