

Introduction

Measurements of nucleic acids in the UV/Vis spectral range in general are used for determination of concentration, estimation of purity and in case of modified nucleic acids calculation of a modification rate.

In standard UV/Vis photometers cuvettes with a pathlength of 1cm are used. The main drawbacks of these setup for nucleic acid measurements are the need of a very high volume (typically 50-1000 μ l, even though most biological assays work below 50 μ l) and the need for sample dilution (typically 1:10-1:100) in most applications in order to be in the linear measurement range of the photometer.

The LabelGuard™ Microliter Cell faces both in an optimized manner. By choosing one of two lids with different pathlengths (1mm and 0.2mm) you get an accurate dilution factor of 1:10 or 1:50 without the need of diluting your sample. Additionally the volumes needed for the measurements are very low for this lids (3 μ l and 1 μ l respectively).

The extremely high reproducibility, avoiding dilution errors, and the possibility to retrieve the samples, are the outstanding features of the LabelGuard™ Microliter Cell.

Quantitation of nucleic acids

For determination nucleic acid concentration in solution the absorbance at wavelength 260 nm (A_{260}) is used. The function describing the concentration to absorbance relation is the Lambert-Beer Law: $A = \epsilon * c * d$.

The absorbance (A) is the product of the substance specific extinction coefficient (ϵ), the concentration of the absorbing sample (c), and the optical pathlength in cm (d).

For the different types of nucleic acid solutions the absorbance - concentration (ng/ μ l) relation in dependence of the pathlength is as follows:

Amount at 1 AU-260 nm / Nucleic Acid Type	1cm Cell Dilution Factor = 1	LabelGuard™ Lidtype 1 1mm pathlength Dilution Factor = 10	LabelGuard™ Lidtype 2 0.2mm pathlength Dilution Factor = 50
dsDNA	50 ng/ μ l	500 ng/ μ l	2500 ng/ μ l
ssDNA	37 ng/ μ l	370 ng/ μ l	1850 ng/ μ l
ssRNA	40 ng/ μ l	400 ng/ μ l	2000 ng/ μ l
Oligo	30 ng/ μ l	300 ng/ μ l	1500 ng/ μ l

Interpretation of purity

Depending on the extraction/purification or synthesis/purification method of the nucleic acids different impurities can be expected (TRIzol, humic acids, carbohydrates, Guanidine thiocyanate, nucleotides, peptides, EDTA, phenol and protein). It is recommended to include OD ratio measurement (A260/A280 and A260/A230) for purity estimation.

Ratio A260/A280

In solution, pure DNA and RNA typically have A260/A280 ratios of 1.8 and 2.0. If the absorbance ratio is significantly less, the nucleic acid is probably contaminated with protein. Accurate quantification of nucleic acid is not reliable without prior purification, and the efficacy of this can be judged by the A260 /A280 ratio.

Ratio A260/A230

For RNA samples the ratio values <2.0 point out contamination with genomic DNA. Successful DNase I treatment displays in ratio values > 2.0.

Ratio values <1.5 indicate impurities of extraction chemicals or incompletely removed constituents of cells.

Note: Both ratio values can also be perturbed easily by pH, even if the nucleic acid samples are clean. Use buffers around 7.5 for your measurements.

Dye incorporation rate

To determine dye incorporation rate the absorbance reading at the wavelength corresponding to the extinction coefficient reported for the dye is used. Use the Lambert-Beer Law as above to determine the dye concentration ($c = A / (\epsilon * d)$). Comparing these value with the DNA concentration gives a dye incorporation rate.

Example: Frequency of Incorporation (FOI) of Cy3 and Cy5 per 1000 bases:

$$\text{FOI}(\text{Cy3}) = 58.5 * \text{A550/A260}$$

$$\text{FOI}(\text{Cy5}) = 35.1 * \text{A650/A260}$$

A430nm is qualified for background correction to eliminate cross talk of fluorescence dyes at A260nm for more accurate dye incorporation rate determination.

Contact information:

For Germany, Austria and all
other regions

IMPLEN GmbH
Wehrlestrasse 33
D – 81679 Munich, Germany
Phone: + 49 (0)89 99 100 583
Fax: + 49 (0)89 14 88 252-117
Email: info@implen.de
Web: www.implen.de

For the UK

Implen UK Ltd.
Cumberland House, 24-28 Baxter Avenue
Southend on Sea, Essex, SS2 6HZ
Phone: +44 (0)1702 335588
Fax: +44 (0)1702 334262
Email: info@implen.co.uk
Web: www.implen.co.uk