



What is nucleic acid quantification (NAQ)?

Sample Preparation

DNA/RNA Purification DNA/RNA Quantification

Application e.g. PCR

Downstream

Data Analysis

of the correct amount of nucleic acid required for downstream applications.

NAQ determines the concentration of DNA or RNA in a sample, enabling the calculation



Why perform NAQ?

Using the correct amount of nucleic acid saves time and money, preventing downstream experimental failures.

Cloning, NGS, qPCR, Ligation, PCR, Transfection, Microarrays, Sanger sequencing

Downstream applications include:

In PCR techniques: Too little NA can result in failure to amplify

- the target
- Too much NA used can lead to nonspecific amplification

and purity to be reported in publication.

MIQE - Minimum information for publication of quantitative real-time PCR experiment

Note: MIQE guidelines require input NA quality

Accurate quantification of NA prior to library

In NGS:

- prep helps obtain high-quality reading data • Insufficient NA will result in low
- sequencing efficiency NA overabundance will result in poor
- quality data

Sensitivity

Things to consider when selecting a tool



What quantity ranges do you expect?



How many samples do you quantify at a time?

Absorbance – UV-Vis **Fluorescence**

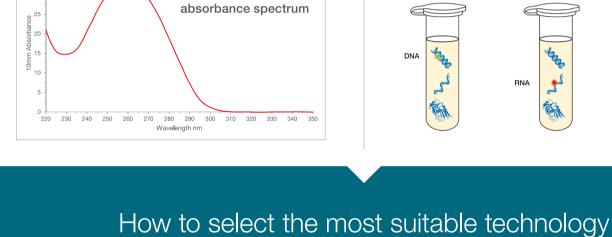
Technologies used in NAQ



Provides concentration of nucleic acid and

protein by measuring the amount of light

that it absorbs. **DNA or RNA**

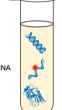




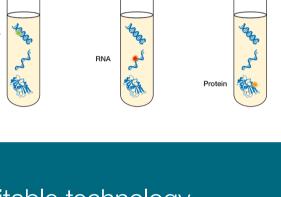
In a fluorometric assay, dyes bind selectively to DNA, RNA, or protein of interest.

DNA dye selectivity RNA dye selectivity Protein dye selectivity









Absorbance Fluorescence

benefits	Limitations	Deficition	Limitations		
Simple	Limited selectivity	Specific	Requires reagents and standards		
Purity	Limited sensitivity	Sensitive	No purity identification		
Contaminant information		Accurate			
? Did you know?					



you cannot rely on the measurement. In this case switching to fluorescence will improve your result.

How solutions from Thermo Fisher Scientific could help you



Absorbance – UV-Vis



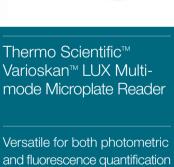


sample flexibility using 96-

More than

150 samples

384-well plates, cuvettes,



and offers increased flexibility

addressing diverse research

needs in laboratories

of one sample at a time

Only 1-2µL sample needed

for accurate quantification

Thermo Scientific™

Spectrophotometers

Nanodrop™

Ultra and Ultra^c

Quantifies 8 samples

at a time for improved

productivity

or the Thermo Scientific™ μDrop™ Plate Increase throughput

LUX

A few samples 10-150 samples

Purified DNA/RNA

Direct absorbance at 260 nm

A pedestal, cuvette, array of pedestals	A μDrop plate	96-well plate	384-well plate		
NanoDrop	Multiskan SkyHigh with μDrop plate	Multiskan SkyHigh	Multiskan SkyHigh	Varioskan I	
Fluorescence*					
Quickit 4		Operation of the state of the s			





A tube/tube strips

Qubit +

Qubit Assay

Qubit™ Flex Fluorometer Quantifies 8 samples with high sensitivity, accuracy and precision

Invitrogen™

LUX Multimode Microplate Reader Versatile for both photometric and fluorescence quantification and offers increased flexibility addressing diverse research needs in laboratories

Thermo Scientific™ Varioskan™

If a 260 nm/280 nm result is not reliable

Increase throughput

A fluorometric assay A few samples Many samples

96-well plate A µDrop plate 384-well plate Varioskan LUX Varioskan LUX + with µDrop plate Quant-iT Assay + Quant-iT Assay

*For fluorescent NA quantification, the Qubit reagents are available for Qubit instruments, and the Invitrogen™ Quant-iT™ reagents for Varioskan LUX microplate readers.