

throughput sensitivity budget

Nucleic acid quantification

Easy choices for RNA and DNA quantification

Introduction

DNA and RNA quantification, generally referred to as nucleic acid quantification, are performed to determine the concentration of DNA or RNA in a sample prior to downstream experiments.

Detecting and quantitating small amounts of nucleic acid is important in a wide variety of biological applications, including quantitative PCR, cloning, and next-generation sequencing (NGS). Accurate and sensitive nucleic acid quantification is critical for NGS, where both underestimation and overestimation of the concentration can affect the quality of sequencing data. Sample purity is also an important factor in accurately calculating the amount of DNA or RNA in a sample.

Two optical detection technologies are commonly used to quantify nucleic acids: spectrophotometry in the ultraviolet-visible (UV-Vis) range, and fluorescence utilizing dyes specific to the target. These methods can differ in sensitivity, throughput, sample volume required, and information obtained about the sample. Choosing the right technology for your samples and workflow can save significant time and money by helping to prevent downstream experimental failures.

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Methods of quantification

UV-Vis technology

Spectrophotometric measurement of nucleic acids is based on the intrinsic absorptivity of nucleic acids. When an absorbance spectrum is measured, nucleic acids absorb light with a characteristic peak at 260 nm. Using the Beer-Lambert equation, the absorbance at 260 nm measured on a spectrophotometer can be used to calculate the concentration of nucleic acids.

UV-Vis spectrophotometers allow absorbance readings of nucleic acids to be taken using a variety of formats, including microvolumes for precious samples, cuvettes for dilute samples, and microplate readers for high-throughput detection. Spectrophotometers can simplify analysis by automatically calculating nucleic acid concentration along with measuring sample purity.

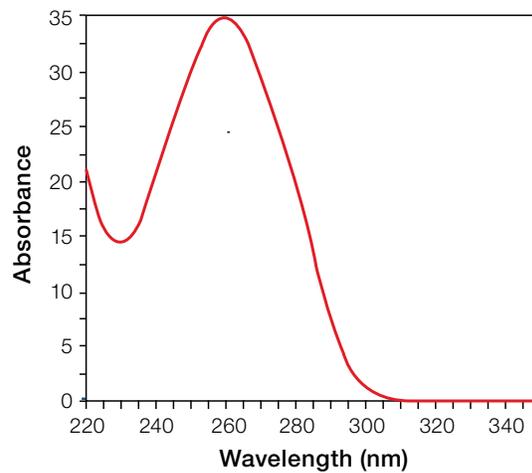


Figure 1. Typical nucleic acid absorbance spectrum.

Detection range: one selection criterion among instruments

	Instrument	dsDNA quantification range (ng/μL)
UV-Vis	NanoDrop One	2–27,500
	NanoDrop One ^c	0.2–27,500
	NanoDrop Eight	2–10,000
	NanoDrop Lite Plus	2–1,500
	Multiskan SkyHigh*	1.1–310
	Varioskan LUX**	0.4–500
Fluorescence	Qubit 4	0.005–4,000
	Qubit Flex	0.005–4,000
	Varioskan LUX†	0.003–4,000

* Multiskan SkyHigh spectrophotometer with 140 μL in 96-well plate.

** Varioskan LUX reader with 140 μL in 96-well plate.

† Varioskan LUX reader measures both UV-Vis and fluorometric signals. Fluorometric range is determined by Quant-iT assay kits.





Fluorescence technology

Sensitivity and specificity are two good reasons to use fluorometers to detect, quantify, and monitor analytes and their reactions in the lab. These instruments measure the intensity of the fluorescent signal from dyes attached to biological molecules as well as naturally fluorescent molecules based on their signature excitation (Ex) and emission (Em) wavelengths. Fluorometers help to simplify fluorescence analysis while minimizing the amount of sample required and saving time.

Fluorometric measurement of nucleic acids is based upon the use of fluorogenic dyes that bind selectively to DNA or RNA. These dyes only emit a fluorescent signal when bound to the target. To measure nucleic acid concentration, a calibration curve is generated using standard samples with a known concentration. An unknown sample concentration is calculated by comparing the relative fluorescence units (RFUs) of the sample to the RFUs of the standards used in calibration. The limit of detection of the measurements is specific to each assay.

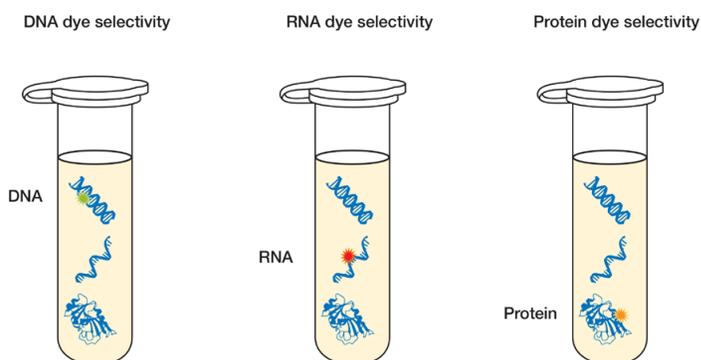


Figure 2. Specificity. Different dyes selectively binding to specific target molecules to be quantified.

Benefits and limitations of quantification technologies

	Spectrophotometry (UV-Vis)	Fluorescence
Benefits	<ul style="list-style-type: none"> • Simple—no sample preparation, dyes, or standards are required • Provides direct measurements of purity ratios—A_{260}/A_{280} and A_{260}/A_{230} • Provides information on contaminants—can identify non-nucleic acid contamination in samples (proteins, phenol, guanidine salts) 	<ul style="list-style-type: none"> • Specific—measurement for dsDNA and RNA according to your needs • Sensitive—can measure pg/mL; recommended method for very dilute nucleic acid samples • Accurate—quantifies target accurately despite contamination being present in the sample, including nucleic acid contaminants
Limitations	<ul style="list-style-type: none"> • Minimal specificity—generally limited in distinguishing between DNA or RNA • Limited sensitivity—detection limits are higher than fluorescence-based methods 	<ul style="list-style-type: none"> • Extra step—additional time is needed for reagent and sample preparation • No purity information—contaminants like proteins, phenol, and guanidine salts are not measured





NanoDrop One and One^C spectrophotometers

Quantify with the NanoDrop One or One^C instrument and analyze with Acclaro technology

Check the quantity and quality of DNA, RNA, and protein with only 1–2 μL of sample in seconds with no dilutions using Thermo Scientific™ NanoDrop™ One and One^C Microvolume UV-Vis Spectrophotometers. Gain a more complete understanding of sample quality before using samples in downstream applications with Thermo Scientific™ Acclaro™ Sample Intelligence technology, which is built into every NanoDrop One instrument. Optional software enables compliance in regulated labs, including 21 CFR Part 11 requirements.

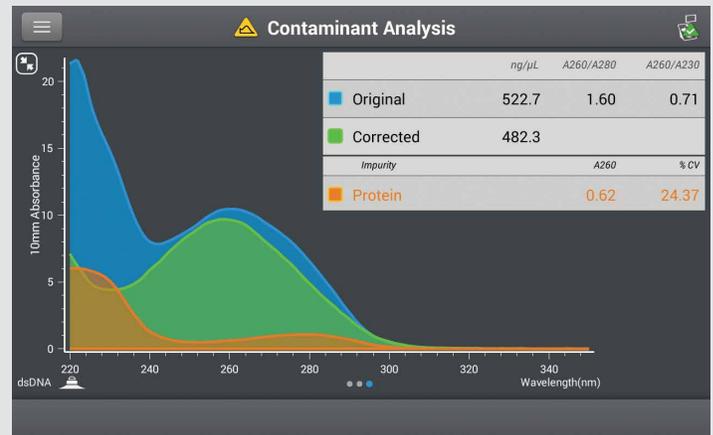
The NanoDrop One^C spectrophotometer adds experimental flexibility and increases the dynamic range. Use cuvettes to measure dilute samples and optical density of bacterial cultures, or to perform kinetic experiments, including with cuvette temperature control and stirring. The cuvette position can be used with the instrument arm up or down.



Identify contaminants in samples with Acclaro technology

Save days of troubleshooting experiments when you make informed decisions on sample suitability for your application. Acclaro technology offers enhanced sample analysis with:

- Contaminant identification and corrected results
- Instant feedback about sample quality with on-demand technical support and guided troubleshooting
- Embedded sensor and digital image analysis that ensures measurement integrity



Acclaro technology detects dsDNA sample contaminated with protein. The absorbance contribution from the protein (orange) is subtracted from the original result (blue) to obtain the corrected dsDNA concentration (green).

Learn more at thermofisher.com/nanodropone





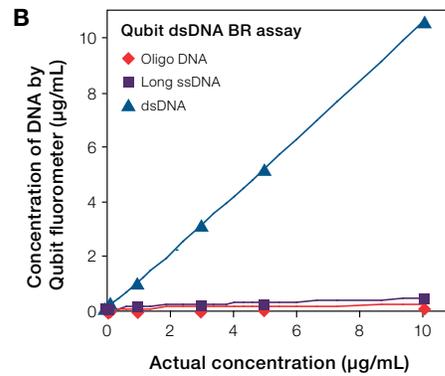
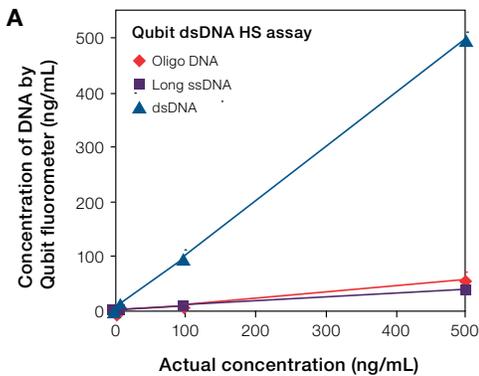
Qubit 4 Fluorometer

Fast and sensitive quantification of DNA, RNA, and protein

The Invitrogen™ Qubit™ 4 Fluorometer is a benchtop microvolume fluorometer designed to accurately measure DNA, RNA, and protein quantity. Whether you are an expert or a novice, the easy-to-use touchscreen menus make it easy to perform assays, with accurate and reliable results displayed in just a few seconds.

Key benefits

- **High sensitivity**—more sensitive than UV absorbance-based quantification
- **Accuracy and speed**—accurately quantifies DNA, RNA, or protein in less than 3 seconds per sample
- **Helpful sample prep calculator**—integrated reagent calculator determines amount of dye and buffer needed
- **Ideal for precious samples**—uses as little as 1 µL of sample
- **Flexible data management**—store up to 1,000 samples internally or export CSV or PDF results to the cloud, USB drive, or computer
- **Portable with small footprint**—small enough to fit in lab coat pocket
- **Optimized reagents and tubes**—Invitrogen™ Qubit™ reagents and tubes are designed to work seamlessly with Qubit fluorometers
- **Intuitive touchscreen**—quickly get to data generation
- **User interface languages available**—English, French, German, Spanish, Italian, Japanese, and Chinese



Use high-sensitivity (HS) assays for low concentrations and broad-range (BR) assays for high concentrations of dsDNA

Figure 3. Detection of double-stranded DNA by the Invitrogen™ Qubit™ dsDNA HS (A) and BR (B) Assay Kits. Duplicate samples of long ssDNA, oligo DNA, or lambda dsDNA at concentrations of 0.5 to 500 ng/mL in the assay tube were quantified using the Qubit dsDNA HS assay, and at concentrations of 0.01 to 10 µg/mL in the assay tube using the Qubit dsDNA BR assay, according to kit protocols.

Learn more at thermofisher.com/qubit4





NanoDrop Eight UV-Vis Spectrophotometer

Higher-throughput sample analysis plus new capabilities to further speed up multi-sample processing

Intelligent analysis, eight samples at a time

Ideal for higher-throughput workflows, the Thermo Scientific™ NanoDrop Eight™ UV-Vis Spectrophotometer measures 8 samples at a time in less than 20 seconds and a 96-well plate in under 6 minutes. Software identifies sample contaminants and reports true sample concentration with Acclaro Sample Intelligence technology, facilitating downstream experiment success and avoiding costly rework.



Key benefits

- Improved productivity with capability of analyzing up to eight 1 μ L samples at one time
- Software provides concentration and identifies and corrects for common impurities found in nucleic acid and protein samples
- Wider dynamic range allows you to measure higher-concentration samples without dilution—up to 10,000 ng/ μ L of dsDNA and 145 mg/mL of IgG
- Pharma ready—optional 21 CFR Part 11-compliant software makes compliance easy, and data output is ready for laboratory information management system (LIMS) integration
- High throughput for environments such as biorepositories, genotyping facilities, and quality control labs
- Improved productivity for busy labs where multiple researchers use a single-sample NanoDrop instrument

New capabilities to speed up your analyses even more

- **Auto-Blank and Auto-Measure features**—streamline multi-sample processing with instant measurements
- **Ergonomic ambidextrous pipetting**—left- and right-handed users can quickly and comfortably dispense samples
- **Sample position illuminator**—LEDs light up to keep measurements on track



Learn more at [thermofisher.com/nanodropeight](https://www.thermofisher.com/nanodropeight)





Qubit Flex Fluorometer

Accurate and sensitive quantification of DNA, RNA, and protein with flexible throughput

With the same convenience and ease of use as the Qubit 4 Fluorometer (p. 7), the Invitrogen™ Qubit™ Flex Fluorometer provides improved throughput. The Qubit Flex instrument can selectively and accurately measure the concentrations of up to 8 samples of DNA, RNA, or protein simultaneously.



Key benefits

- **High sensitivity**—more sensitive than UV absorbance-based quantification
- **Accuracy and speed**—accurately quantifies DNA, RNA, or protein in less than 3 seconds per sample
- **Helpful sample prep calculators**—integrated calculators help determine the amount of dye and buffer needed
- **Specialized calculators for NGS workflows**—easily determine molarity and normalize sample concentrations
- **Ideal for precious samples**—uses as little as 1 µL of sample
- **Plenty of data storage**—stores data for up to 10,000 samples internally
- **Flexible data export**—as CSV or PDF to network drive, cloud, or USB drive
- **Flexible throughput**—measure up to 8 samples per run
- **Portable with small footprint**—easy to move and store on shelves in the lab
- **21 CFR Part 11 ready**—available with optional software to support electronic record keeping
- **Intuitive touchscreen**—quickly get to data generation

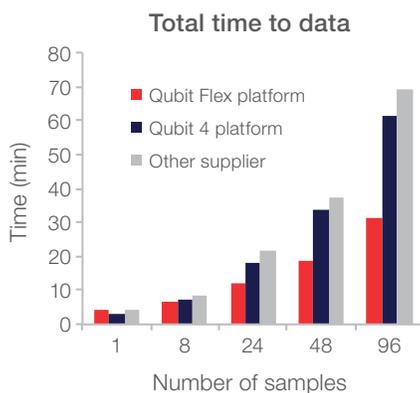


Figure 4. Time trial on 1 to 96 samples shows up to 50% time savings with the Qubit Flex Fluorometer. Time savings was first observed after running 8 samples, with more time saved when measuring even more samples.

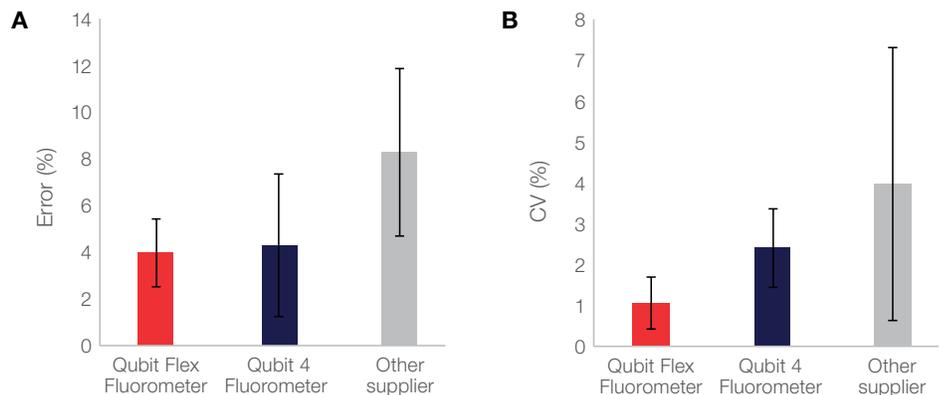


Figure 5. Qubit instruments deliver better accuracy and precision. The Qubit dsDNA BR and 1X dsDNA HS Assay Kits were run with 4 DNA sample concentrations (HS: 0.1, 1, 5, and 10 ng/µL; BR: 2, 20, 50, and 100 ng/µL) in 8 replicates on the Qubit 4 Fluorometer, Qubit Flex Fluorometer, and another supplier's fluorometer. The percent relative error (lower is more accurate) and CV (lower is more precise) were determined for each concentration and averaged across all concentrations for each instrument.

Learn more at thermofisher.com/qubitflex





Multiskan SkyHigh Microplate Spectrophotometer

Flexible medium- to high-throughput UV-Vis detection of nucleic acids from microvolumes to microplates to cuvettes

The monochromator-based Thermo Scientific™ Multiskan™ SkyHigh Microplate Spectrophotometer offers high throughput and sample flexibility for UV-Vis nucleic acid detection using 6- to 48-, 96-, and 384-well plates, cuvettes, or the Thermo Scientific™ μ Drop™ Plate. With a user-friendly touchscreen interface, all users can easily set up their assays using the prebuilt sessions for nucleic acid quantification.



Key highlights

- Ready-made sessions for quantification of dsDNA, ssDNA, and RNA, as well as custom nucleic acid sequences
- Allows for virtually any other type of photometric assay, including colorimetric and scattering assays
- No hassle when using different sample volumes in microplates: pathlength correction (PLC) will be performed automatically for correct concentration calculations, without any need for user input



Varioskan LUX Multimode Microplate Reader

Our most versatile microplate reader for performing photometric and fluorescence quantification of nucleic acids

The Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader supports photometric, fluorescence, luminescence, time-resolved fluorescence (TRF), and AlphaScreen™ technologies to address diverse research needs using microplate detection. Despite the instrument's optical system complexity, this instrument offers simplicity with top-of-the-line performance. It has automated dynamic range (ADR) selection, which adjusts the optimal reading range based on signal intensities, thus preventing signal saturation. This is critical when measuring high and low nucleic acid concentrations in one single plate.



Key highlights

- Ideal for running all Invitrogen™ Quant-iT™ assays using a double monochromator optical system without any need for filters
- Optimal fluorescence signals can be always measured using ADR, which completely avoids manually setting the gain values
- Safety checks alert users of potential problems before they happen
- Instrument can be configured to meet exact research needs and later upgraded if needed

Learn more at [thermofisher.com/multiskanskyhigh](https://www.thermofisher.com/multiskanskyhigh) and [thermofisher.com/varioskanlux](https://www.thermofisher.com/varioskanlux)



Skant Software

Key highlights

- Two editions available—Research Edition for life sciences, and Drug Discovery Edition with features for compliance with 21 CFR Part 11
- Open-license software for unlimited installation on multiple computers
- Searchable Thermo Scientific™ Skant™ Cloud Library with ready-to-use protocols that include calculations
- Robotic automation interface
- Measurement sessions are easy to set up and navigate
- Virtual Pipetting Tool assists in defining the plate layout
- Single- or multiple-wavelength measurements, as well as multi-technology measurements
- Capable of endpoint, kinetic, spectral scanning, bottom-reading with multipoint option, and kinetic-spectral measurements
- Built-in calculations for fast, accurate data analysis, including:
 - Parallel line analysis
 - Enzyme kinetic analysis of K_m and V_{max}
 - Z-factor
 - Linear and logistic curve fits with extrapolation
- Data transferable to a Microsoft™ Excel™ file with a single click
- User interface languages available—English, French, German, Spanish, Portuguese, Italian, Russian, Japanese, and Chinese



Boost productivity and prevent errors with Skant Software 7.0

Thermo Scientific™ Skant™ Software helps you optimize your research with an easy-to-read visual workflow, intuitive data analysis, and flexible exporting capabilities. Logical software structure reduces the need for training and makes it simple to create a new protocol adapted to different experimental conditions. Its high processing speed provides the ability to analyze your data on a different computer than the main lab computer. With Skant Software, you have full control over the instrument settings for all your Thermo Scientific™ microplate readers.

The special Drug Discovery Edition uses an integrated database system for maximal security with features that enable 21 CFR Part 11 compliance, like digital signatures, user login with Microsoft™ Windows™ authentication, and complete audit trails. Both Research and Drug Discovery editions can be installed on the same computer simultaneously, and experimental protocols can be freely shared between them. Full security and integrity on the error-preventing platform of Skant Software help to simplify both data acquisition and analysis, facilitating an effortless workflow so that you can push your research forward with confidence.

Learn more at thermofisher.com/skant



Product listings

Nucleic acid quantification instruments

Instrument	More info	Cat. No.
NanoDrop One Microvolume UV-Vis Spectrophotometer	thermofisher.com/nanodrop	ND-ONE-W or ND-ONE**
NanoDrop One ^o Microvolume UV-Vis Spectrophotometer		ND-ONEC-W or ND-ONEC**
NanoDrop Lite Plus Microvolume UV-Vis Spectrophotometer		NDLPLUSGL
NanoDrop Eight UV-Vis Spectrophotometer		NDE-GL
Multiskan SkyHigh Microplate Spectrophotometer*	thermofisher.com/platereader	A51119500C
Varioskan LUX Multimode Microplate Reader*		VL0000D0
Qubit 4 Fluorometer	thermofisher.com/qubit	Q33238
Qubit Flex Fluorometer		Q33327

* Different models available; visit web pages for more information.

** Cat. No. is region-specific.

Find RNA and DNA nucleic acid quantification information at thermofisher.com/naq

For use with Qubit 4 and Qubit Flex Fluorometers

Qubit assay	Target	Cat. No.
Qubit 1X dsDNA HS Assay Kit	dsDNA	Q33230
Qubit dsDNA HS Assay Kit	dsDNA	Q32851
Qubit 1X dsDNA BR Assay Kit	dsDNA	Q33265
Qubit dsDNA BR Assay Kit	dsDNA	Q32850
Qubit ssDNA Assay Kit	ssDNA	Q10212
Qubit RNA HS Assay Kit	RNA	Q32852
Qubit RNA BR Assay Kit	RNA	Q10210
Qubit RNA XR Assay Kit	RNA	Q33223
Qubit miRNA Assay Kit	MicroRNA	Q32880
Qubit RNA IQ Assay Kit	RNA	Q33221

Get more information on Qubit assays at thermofisher.com/qubitassays

Quant-iT bulk reagents and kits for >2,000 samples

Quant-iT assay	Target	Cat. No.
Quant-iT PicoGreen dsDNA Assay Kit	dsDNA	P7589
Quant-iT PicoGreen dsDNA Reagent	dsDNA	P7581
Quant-iT OliGreen ssDNA Assay Kit	ssDNA	O11492
Quant-iT OliGreen ssDNA Reagent	ssDNA	O7582
Quant-iT RiboGreen RNA Assay Kit	RNA	R11490
Quant-iT RiboGreen RNA Reagent	RNA	R11491

Quant-iT assays and reagents are compatible with the Fluoroskan and Varioskan LUX or other fluorescence-reading microplate readers.

Quant-iT assay kits and reagents for 20 to 2,000 samples

Quant-iT assay	Target	Cat. No.
Quant-iT 1X dsDNA HS Assay Kit	dsDNA	Q33232
Quant-iT dsDNA HS Assay Kit	dsDNA	Q33120
Quant-iT 1X dsDNA BR Assay Kit	dsDNA	Q33267
Quant-iT dsDNA BR Assay Kit	dsDNA	Q33130
Quant-iT RNA Assay Kit	RNA	Q33140
Quant-iT RNA BR Assay Kit	RNA	Q10213
Quant-iT RNA XR Assay Kit	RNA	Q33225

Quant-iT assays and reagents are compatible with the Fluoroskan and Varioskan LUX or other fluorescence-reading microplate readers.

Get more information on Quant-iT assays and reagents at thermofisher.com/quantit

 Find solutions that measure up at thermofisher.com/naq