

invitrogen



Nucleic acid quantification

Qubit fluorometers and assays

Accurate, specific, and sensitive quantification of DNA, RNA, and protein samples

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part of Thermo Fisher Scientific

Qubit fluorometers

Intuitive user interface coupled with accurate measurements

Invitrogen™ Qubit™ 4 and Qubit™ Flex Fluorometers are benchtop microvolume fluorometers designed to accurately measure DNA, RNA, or 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. Both instruments provide flexible data exportation using a USB drive, Wi-Fi cloud connectivity, or direct USB cable connection so your quantification data are easily accessed.

Key benefits of Qubit fluorometers

- **High sensitivity**—more sensitive than UV absorbance-based quantification
- **Accuracy and speed**—accurately quantifies DNA, RNA, or protein in less than 3 seconds
- **Ideal for precious samples**—requires as little as 1 μL of sample
- **Optimized reagents and tubes**—Invitrogen™ Qubit™ reagents and assay tubes work best with Qubit fluorometers



Figure 1. Qubit Flex and Qubit 4 Fluorometers with intuitive touchscreens and applications for specific assays.

Qubit fluorescence technology

Qubit fluorometers and assay kits are designed to measure the intensity of the signal from fluorescent dyes bound to specific biological molecules. These optimized dyes bind selectively to DNA, RNA, or protein and only emit a fluorescent signal when bound to the target.

Qubit fluorometers use specialized curve-fitting algorithms to develop a calibration curve using standard samples with a known concentration. An unknown sample concentration of DNA, RNA, or protein is calculated by comparing the relative fluorescence units (RFUs) of the sample to the RFUs of the standards used in calibration. The detection limits of the measurements are specific to each assay.

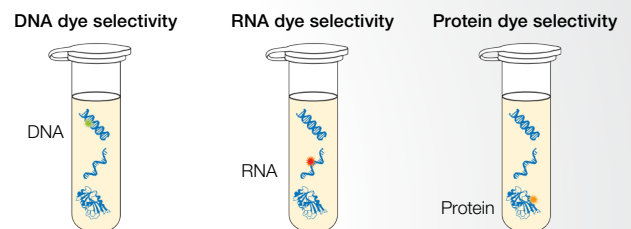


Figure 2. Fluorescent dyes selectively bind to DNA, RNA, or protein. Dyes only emit signal when bound to the target.

Convenient, easy-to-use onboard calculators

Reagent calculator

The reagent calculator conveniently calculates how much working solution to prepare based on the number of samples to quantify. Available in both the Qubit 4 and Qubit Flex Fluorometer models.

Assay range calculator

The assay range calculator displays the core sample concentration range based on the sample volume, as well as the extended low and high ranges. This aids in the selection of the appropriate Qubit assay for the most accurate quantification based on your sample volume and estimated sample concentration. This calculator is only available with the Qubit Flex Fluorometer.

Reagent Calculator

How many samples? 15

How many standards? 2
(0, 2 or 3 standards)

Include overage
(3 extra tubes)

Results:

Add 34µL dye to 6766µL buffer for a total volume of 6800µL

Done

Assay Range Calculator

Enter sample volume used:
1X dsDNA HS

10 µL

Range	Sample Concentration (ng/µL)
Extended low	0.01 - < 0.02
Core	0.02 - 10
Extended high	> 10 - 12

0.02 - 10

Done

Figure 3. Reagent and assay range calculators. Easily make working solutions for all assays that are not in 1X format using the reagent calculator. The assay range calculator aids in the determination of sample volume requirements based on required accuracy.

Calculators for next-generation sequencing (NGS) workflows

Molarity calculator

Quickly calculate the molarity of your samples based on nucleic acid length and the measured concentration. The molarity calculator is only available on the Qubit Flex Fluorometer.

Normalization calculator

Easily normalize to a desired mass, concentration, or molarity with the normalization calculator. This replaces spreadsheet calculations for standard normalization during library preparation for sequencing. The normalization calculator is only available on the Qubit Flex Fluorometer.

Molarity Calculator

Desired units: ng/µL to nM

Molecular weight: 660 g/mol

Auto-populate DNA length

Sample	Concentration (ng/µL)	Length (bp)	Molarity (nM)
S1	4.93	500	14.9
S2	4.89	500	14.8
S3	4.89	500	14.8
S4	4.87	500	14.8

Export Cancel Calculate

Normalization Calculator

Final Concentration: 2 ng/µL Final Volume: 10 µL

Sample	Add sample (µL)	Add buffer (µL)
S1	2.3	7.7
S2	2.2	7.8
S3	2.3	7.7
S4	2.2	7.8
S5	2.2	7.8
S6	2.3	7.7
S7	2.2	7.8
S8	2.3	7.7

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Export Done

Figure 4. Integrated post-results molarity and normalization calculators. Use the molarity calculator to convert values to molarity based on nucleic acid length. Use the normalization calculator to determine how to dilute the samples to the same concentration.

Personalized workflows

Envision and create custom assays for the Qubit 4 Fluorometer

MyQubit functionality brings your favorite fluorescence assays right to your benchtop, providing a reliable platform for many quantitation needs—from laboratory research and quality control to process monitoring and beyond. Any fluorescent reagent or assay that is spectrally compatible with the Qubit hardware can be adapted for use with the Qubit 4 Fluorometer.

Compare Qubit fluorometers

	Qubit 4 Fluorometer	Qubit Flex Fluorometer
Sample throughput	1 sample in 3 seconds	1 to 8 samples in 3 seconds
User interface	5.7 in. color touchscreen	8 in. color touchscreen
Onboard calculators	Reagent calculator	Reagent calculator Assay range calculator Molarity calculator Normalization calculator
Informs where the sample concentration resides within the assay range	Provides quantification data for samples that are within the core and the extended range of the standard curve. Sample concentrations that are out of range are not given a measurement.	
System check	Qubit 4 System Verification Assay Kit	Qubit Flex System Verification Assay Kit
Fluorometer mode	Yes	No
Programable open format	Yes—MyQubit	No
Instrument footprint (W x L x H)	13.6 x 25 x 5.5 cm 5.4 x 10 x 2.2 in.	1.86 x 28.2 x 10.3 cm 7.3 x 11.1 x 4.1 in.
Sample data storage	1,000 samples	10,000 samples
Data export	Wi-Fi USB drive Direct to computer via USB or ethernet cable	Wi-Fi USB drive Direct to computer via USB or ethernet cable
Light sources	Blue LED (peak ~470 nm), Red LED (peak ~635 nm)	Blue LED (peak ~460–480 nm), Red LED (peak ~620–640 nm)
Excitation filters	Blue LED (430–495 nm), Red LED (600–645 nm)	Blue LED (456–484 nm), Red LED (612–644 nm)
Emission filters	Green (510–580 nm), Red (665–720 nm)	Green (513–563 nm), Far-red (671–693 nm)

21 CFR Part 11 compliance support software

The Qubit Flex Fluorometer combines high-performance nucleic acid and protein quantifications with regulatory compliance support for busy labs that need efficient processes. Benefits include:

- **High performance**—get accurate and reproducible quantification in seconds
- **Intuitive interface**—simple to use, with several convenient calculators
- **Security, audit, and e-signature (SAE) software**—for 21 CFR Part 11 compliance support of electronic record keeping
- **Multiple assays**—Invitrogen™ Qubit™ DNA, RNA, and protein quality and integrity assays are available

Qubit 4 and Qubit Flex kits for system verification

The Invitrogen™ Qubit™ 4 System Verification Assay Kit and the Invitrogen™ Qubit™ Flex System Verification Assay Kit are fast, easy-to-use, reagent-based assays that test the performance of Qubit fluorometers. Each kit consists of three components: a blank reagent solution, a green fluorescent reagent, and a far-red fluorescent reagent. Paired with a hardware functionality test, the assay is designed to test the internal components of the instrument to help ensure proper functionality.

Qubit RNA quantification assays

There are three RNA assay kits, which offer differing detection ranges, and one microRNA assay kit:

- **Invitrogen™ Qubit™ RNA HS Assay Kit**—high sensitivity
- **Invitrogen™ Qubit™ RNA BR Assay Kit**—broad range
- **Invitrogen™ Qubit™ RNA XR Assay Kit**—extended range
- **Invitrogen™ Qubit™ microRNA Assay Kit**—highly selective for miRNA over rRNA or large mRNA (>1,000 bp)

The RNA assays are accurate for initial sample concentrations from as little as 250 pg/μL to 10,000 ng/μL. These kits are highly selective for RNA over dsDNA. Unlike other RNA assays, they do not require DNase if DNA is present in the sample for an accurate measurement.

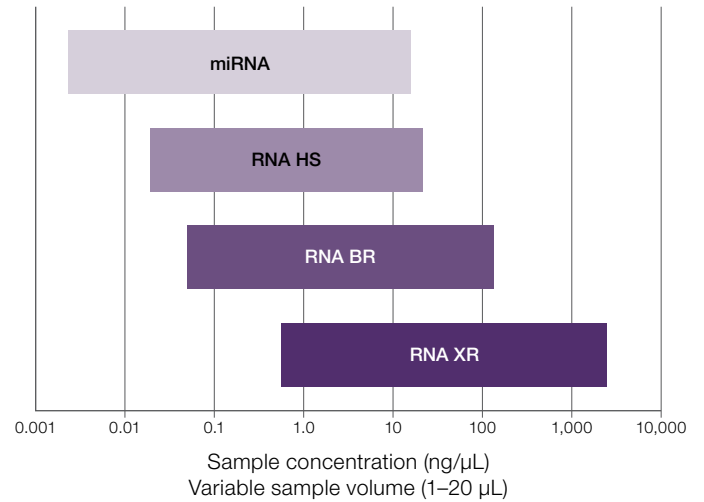


Figure 5. Quantification ranges of Qubit RNA assay kits.

Qubit RNA integrity and quality (IQ) assay

The Invitrogen™ Qubit™ RNA IQ Assay was developed to quickly assess the quality and integrity of an RNA sample. This assay allows assessment of RNA quality at a lower cost and with an easy-to-use, intuitive workflow.

The Qubit RNA IQ Assay utilizes two unique dyes—one that binds to large, intact, and/or structured RNA, and another that selectively binds to small, degraded RNA. Together, they are able to quickly assess the quality and integrity of an RNA sample. To use, simply add your samples to the Qubit RNA IQ working solution, then measure on the Qubit 4 or Qubit Flex Fluorometer.

Results are presented as a total value for the RNA sample integrity and quality, or RNA IQ number, and as the calculated percentage of large and small RNA in the sample. The RNA IQ number is based on a scale of 1 to 10, wherein a high IQ number indicates the majority of the sample consists of large and/or structured RNA. Conversely, a small IQ number indicates the sample comprises mainly small RNA with limited tertiary structure.



Figure 6. A proprietary algorithm is used to report a quality score representative of the ratio of small and large and/or structured RNA in the sample.

Qubit DNA quantification assays

Invitrogen™ Qubit™ DNA assay kits are broadly categorized as double-stranded DNA (dsDNA) assays or single-stranded DNA (ssDNA) assays.

Qubit dsDNA assay kits—available in two detection ranges and two formats

Detection ranges: high-sensitivity and broad-range assays

- The Invitrogen™ Qubit™ dsDNA High-Sensitivity (HS) Assay Kit is for samples with a low concentration of dsDNA, making it ideal for precious samples; it has a detection range between 0.1 and 120 ng
- The Invitrogen™ Qubit™ dsDNA Broad-Range (BR) Assay Kit is ideal for a broad range of DNA concentrations and applications; it can detect between 4 and 4,000 ng

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

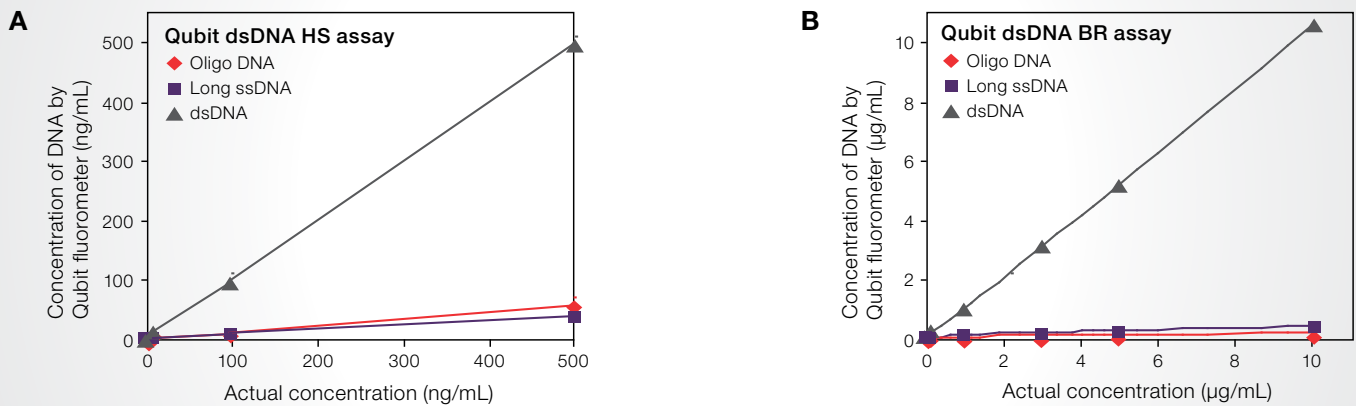


Figure 7. Detection of double-stranded DNA by the Qubit dsDNA HS (A) and BR (B) Assay Kits. Duplicate samples of long ssDNA, oligo DNA, or lambda dsDNA at concentrations of 0.5 to 500 ng/mL in the assay tube were quantified using the Qubit dsDNA HS assay, and at concentrations of 0.01 to 10 µg/mL in the assay tube using the Qubit dsDNA BR assay according to kit protocols.

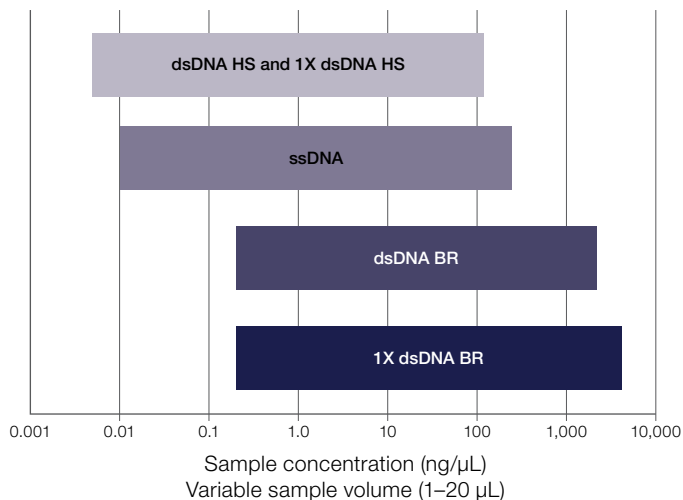


Figure 8. Quantification ranges of Qubit DNA assay kits.

Formats: standard assay and 1X assay

- Invitrogen™ Qubit™ standard assays require same-day mixing of the buffer with the reagent to create the working solution prior to preparing standards and samples for quantification
- Invitrogen™ Qubit™ 1X assays eliminate the step of preparing the working solution
 - The Invitrogen™ Qubit™ 1X dsDNA HS Assay Kit provides the same dynamic range and limit of detection as the standard assay, while the Invitrogen™ Qubit™ 1X dsDNA BR Assay Kit has a wider dynamic range than the standard assay, achieving 4,000 ng/μL in the extended range
 - This format offers a simplified workflow while reducing the tubes in the kit, therefore reducing the amount of plastic used
 - Simply add your sample or standard to the premixed solution, incubate, and read your results

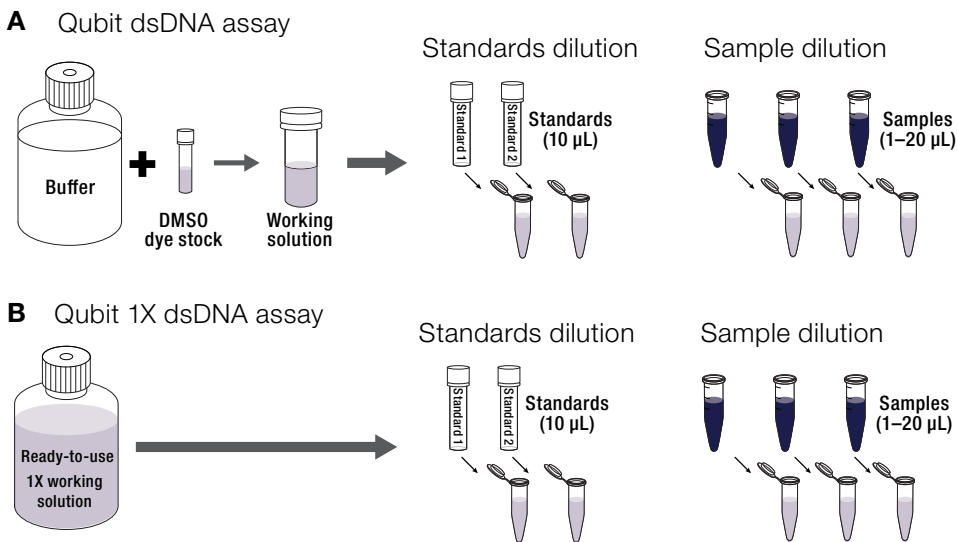


Figure 9. Workflow comparison for the (A) Qubit dsDNA and (B) Qubit 1X dsDNA assays. Standard Qubit dsDNA High Sensitivity (HS) and Qubit dsDNA Broad Range (BR) Assay Kits include a fluorogenic dye, buffer, and dsDNA standards. Prior to each assay, a fresh aqueous working solution needs to be prepared by diluting the dye stock in the provided buffer in a 1:200 ratio. Qubit 1X dsDNA Assay Kits eliminate this step by providing a ready-to-use working solution.

Qubit ssDNA and oligos quantification assays

The Invitrogen™ Qubit™ ssDNA Assay Kit is ideal for quantifying single-stranded DNA or oligonucleotides. It is accurate for initial sample concentrations from 50 pg/μL to 200 ng/μL, providing an assay range of 1–200 ng.

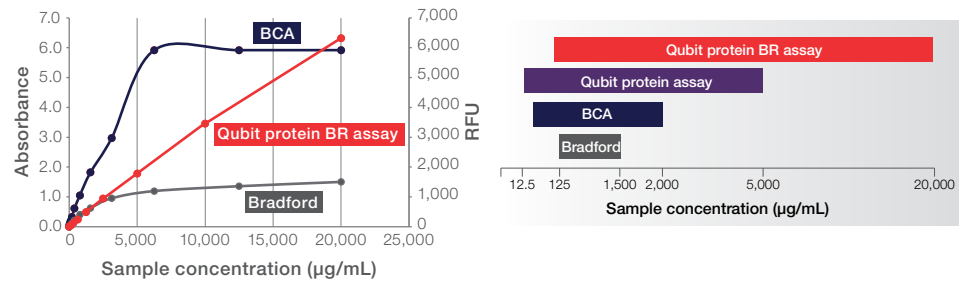
Qubit assays

Qubit assays are designed to work with Qubit fluorimeters. Common contaminants such as salts, free nucleotides, RNA, solvents, detergents, and proteins are well tolerated in Qubit assays.



Qubit protein quantification assays

Invitrogen™ Qubit™ Protein Assay Kits are designed to make protein quantification easy and fast. The assays provide low protein variability, rapid quantitation, and high sensitivity. Common contaminants, such as reducing reagents (DTT, β-mercaptoethanol), salts, free nucleotides, amino acids, solvents, DNA, and detergents (Invitrogen™ Qubit™ Protein BR Assay only), are well tolerated in the assays. The assays' wide dynamic ranges make it easy to determine the concentrations of a wide range of samples compared to standard colorimetric protein assays.



	Qubit Protein BR Assay	Qubit Protein Assay
Platform	Qubit 4 Fluorometer	Qubit 4 and Qubit Flex Fluorometers
Compatibility	Detergents, reducing agents	Reducing agents
Quantitation range	100 µg/mL to 20 mg/mL	12.5 µg/mL to 5 mg/mL

Figure 10. Quantitation ranges of protein assays.

Qubit endotoxin detection assay

The Invitrogen™ Qubit™ Endotoxin Detection Assay Kit is a sensitive and easy-to-use fluorescent endpoint assay that uses amoebocyte lysates to quantify endotoxin in protein, peptides, antibodies, or nucleic acid samples. Amoebocyte lysates are widely used as a simple and sensitive assay for the detection of endotoxin lipopolysaccharide (LPS), the membrane component of gram-negative bacteria.

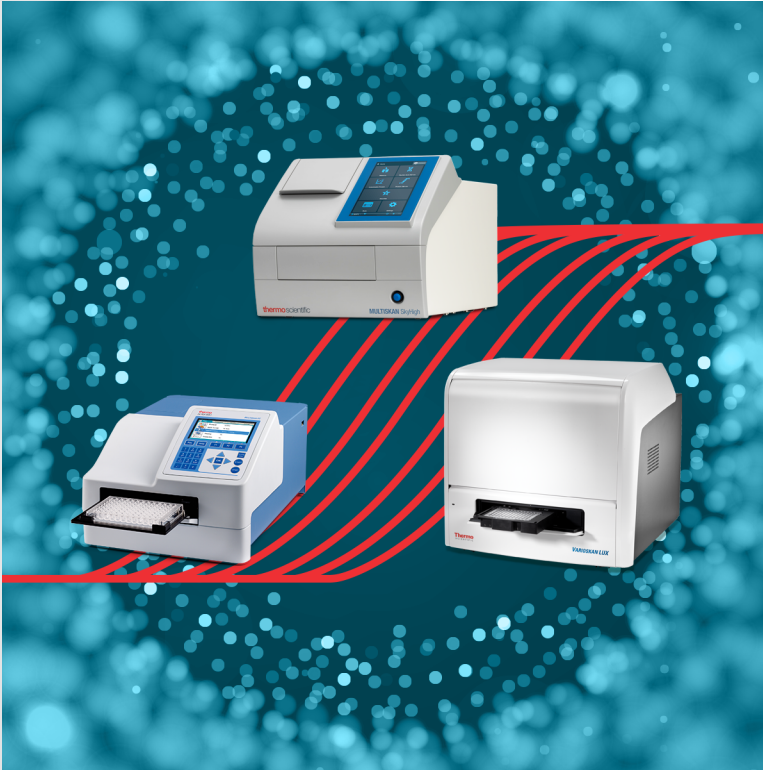
When endotoxin encounters the amoebocyte lysate, a series of enzymatic reactions result in the activation of Factor C, Factor B, and pro-clotting enzyme. The activated enzyme catalyzes a cleavage event in the substrate to produce a strong fluorescent signal. After stopping the reaction, the resulting signal is measured on the Qubit Flex Fluorometer. This fluorescent signal is proportional to the endotoxin concentration in the sample.

Key features and benefits of the Qubit Endotoxin Detection Assay Kit include:

- **Highly sensitive with a broad range**—detect as little as 0.01–10.0 EU/mL
- **Suitable for a wide range of samples**—including proteins, plasmid preparations, DNA, and RNA
- **Easy to use**—when paired with the Qubit Flex Fluorometer, calculations are performed automatically, reducing the potential for error

Determining endotoxin levels is important to assess the efficiency of endotoxin removal methods and prevent endotoxic shock, inflammation, and/or sepsis in tissue culture cells and animals injected with endotoxin-contaminated proteins.

Need higher throughput for your nucleic acid or protein samples?



Qubit assays are ideal when the number of samples you measure at one time is low enough not to warrant a microplate reader. With larger sample batches requiring a fluorescence microplate reader, use Invitrogen™ Quant-iT™ assay kits and reagents, which are designed to use with microplate readers for nucleic acid or protein quantification.

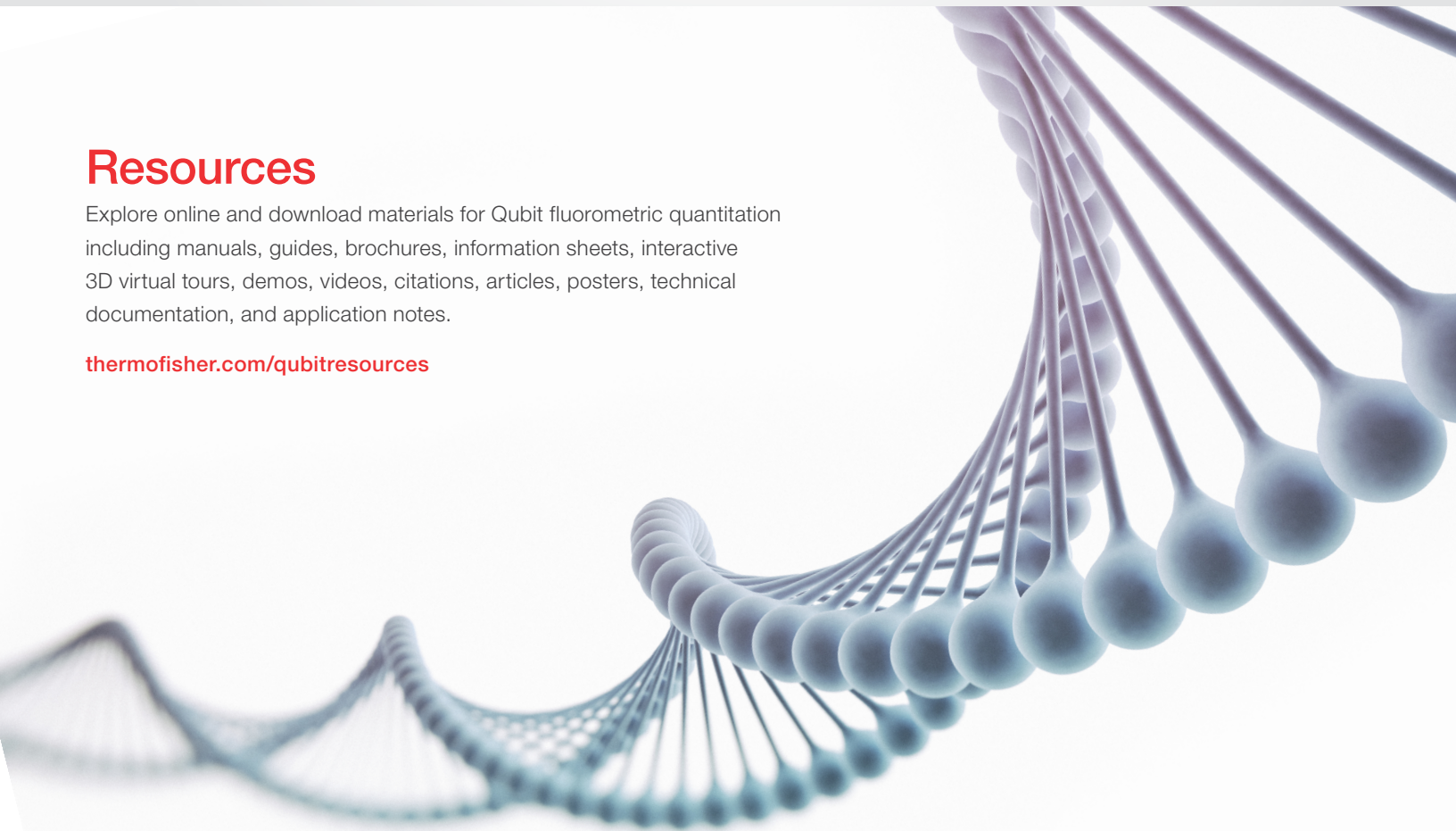
Learn more about Quant-iT assays at thermofisher.com/quantit

Learn more about microplate readers at thermofisher.com/platereaders

Resources

Explore online and download materials for Qubit fluorometric quantitation including manuals, guides, brochures, information sheets, interactive 3D virtual tours, demos, videos, citations, articles, posters, technical documentation, and application notes.

thermofisher.com/qubitresources



Frequently asked questions

Q. I already have a Thermo Scientific™ NanoDrop™ instrument; why should I use a Qubit fluorometer?

A. NanoDrop instruments use UV absorbance to measure DNA and RNA concentrations. Absorbance-based measurements have limitations in distinguishing between DNA, RNA, and free nucleotides, which absorb at 260 nm.

Qubit assays are fluorescence-based. They are designed to only quantify the target analyte. Additionally, fluorescence-based nucleic acid quantification provides a more sensitive dynamic range than absorbance-based instruments.

When used with Qubit assays, Qubit fluorometers can accurately measure low concentrations of sample, while NanoDrop spectrophotometers can detect the presence of common contaminants.

Q. Do I have to use new standards every time?

A. For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can determine the level of comfort you have using the calibration data stored from the last time the instrument was calibrated. We do recommend running a new calibration curve every time you prepare a new working solution.

Q. Is there a difference in signal between supercoiled and relaxed plasmid DNA when using a Qubit fluorometer?

A. Yes, we have seen a 20–30% difference. For the different forms of plasmid DNA, we recommend using a standard that more closely represents the composition of the plasmid DNA in the sample.

Q. Does the Qubit protein assay or Qubit protein BR assay work well in the presence of detergents?

A. The Qubit protein BR assay is compatible with samples that contain up to 5% detergents. The Qubit protein assay is not recommended if detergent is present.

Q. Why are some of the instrument setting menu options not available on my Qubit 4 or Qubit Flex device?

A. To adhere to cybersecurity legal standards, the latest firmware for Qubit 4 and Qubit Flex Fluorometers will require users to log in to access certain menu options (such as instrument settings, software update, and system verification). When not logged in to a user profile, these menu options will appear to be inactive.

Q. Is there a way to verify that my Qubit 4 or Qubit Flex Fluorometer is functioning properly?

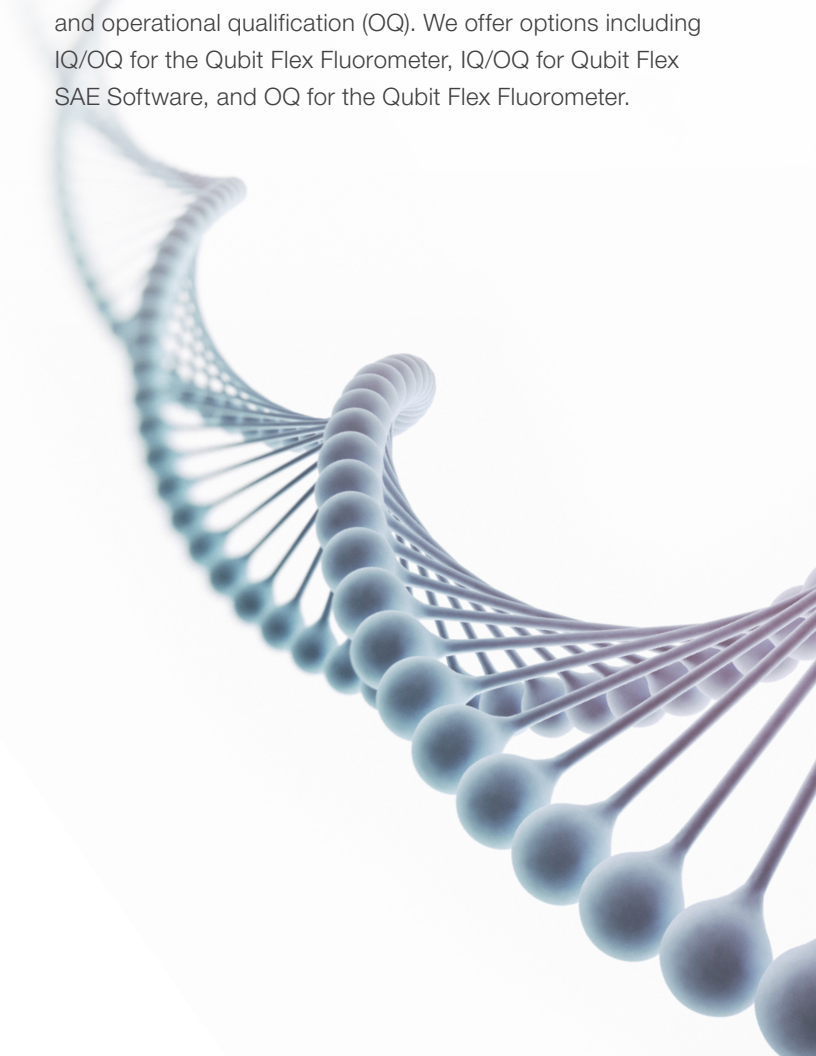
A. Qubit 4 and Qubit Flex System Verification Assay Kits offer a fast, easy-to-use, reagent-based method to test the internal components of a Qubit fluorometer. Perform the system verification when a problem with the instrument is suspected. It is not necessary to perform verification regularly.

Q. How long does the lamp last?

A. There are two LED light sources in the Qubit 4 and Qubit Flex Fluorometers. They are expected to last 5 years.

Q. Do Qubit fluorometers offer qualification services?

A. Yes, our manufacturer-trained and -certified field service engineers (FSEs) can perform installation qualification (IQ) and operational qualification (OQ). We offer options including IQ/OQ for the Qubit Flex Fluorometer, IQ/OQ for Qubit Flex SAE Software, and OQ for the Qubit Flex Fluorometer.



Ordering information

Instruments and accessories	Quantity	Cat. No.
Qubit 4 Fluorometer		
Qubit 4 Fluorometer (w/ Wi-Fi)	1 instrument	Q33238
Qubit 4 Quantitation Starter Kit (w/ Wi-Fi)	1 kit	Q33239
Qubit 4 NGS Starter Kit (w/ Wi-Fi)	1 kit	Q33240
Qubit 4 RNA IQ Starter Kit (w/ Wi-Fi)	1 kit	Q33241
Qubit 4 Protein BR Assay Starter Kit (w/ Wi-Fi)	1 kit	A51292
Qubit Assay Tubes	500 tubes	Q32856
Qubit 4 System Verification Assay Kit	50 assays	Q33237
Qubit Flex Fluorometer		
Qubit Flex Fluorometer	1 instrument	Q33327
Qubit Flex NGS Starter Kit	1 kit	Q45893
Qubit Flex Quantitation Starter Kit	1 kit	Q45894
Qubit Flex Assay Tube Strips	125 tube strips	Q33252
Qubit Flex Assay Reservoirs	100 reservoirs	Q33253
Qubit Flex System Verification Assay Kit	25 assays	Q33254
Qubit Flex Fluorometer with SAE Software for 21 CFR Part 11 Support	1 package	Q45895
Qubit Flex SAE Software for 21 CFR Part 11 Support	1 license	Q31994

Product	Initial sample concentration	Quantitation range	Quantity	Cat. No.
DNA quantification assays				
dsDNA HS assays				
Qubit 1X dsDNA HS Assay Kit	0.005–120 ng/μL	0.1–120 ng	100	Q33230
			500	Q33231
Qubit dsDNA HS Assay Kit	0.005–120 ng/μL	0.1–120 ng	100	Q32851
			500	Q32854
Qubit 1X dsDNA HS Assay Lambda Standard	-	-	-	Q33233
dsDNA BR assays				
Qubit 1X dsDNA BR Assay Kit	0.2–4,000 ng/μL	4–4,000 ng	100	Q33265
			500	Q33266
Qubit dsDNA BR Assay Kit	0.2–2,000 ng/μL	4–2,000 ng	100	Q32850
			500	Q32853
Qubit 1X dsDNA BR Assay Lambda Standards	-	-	-	Q33263
ssDNA and oligos assay				
Qubit ssDNA Assay Kit	0.05–0.2 ng/μL	1–200 ng	100	Q10212
RNA quantification assays				
Qubit RNA HS Assay Kit	250 pg/μL and 100 ng/μL	5–100 ng	100	Q32852
			500	Q32855
Qubit RNA BR Assay Kit	1 ng/μL to 1 μg/μL	20–1,000 ng	100	Q10210
			500	Q10211
Qubit RNA XR Assay Kit	10 ng/μL and 10,000 ng/μL	200–10,000 ng	100	Q33223
			500	Q33224
Qubit microRNA Assay Kit	50 ng/mL to 100 μg/mL	1–1,000 ng	100	Q32880
			500	Q32881

Ordering information (continued)

Product	Quantity	Cat. No.
RNA IQ assays		
Qubit RNA IQ Assay Kit	75 assays	Q33221
	275 assays	Q33222
Qubit RNA IQ Standards	1 set	Q33235

Product	Fluorometer	Initial sample concentration	Quantity	Cat. No.
Protein assays				
Qubit Protein Assay Kit	Qubit Flex, Qubit 4	12.5 µg/mL to 5 mg/mL	100	Q33211
			500	Q33212
Qubit Protein BR Assay Kit	Qubit 4	100 µg/mL to 20 mg/mL	100	A50668
			500	A50669

Product	Fluorometer	Quantity	Cat. No.
Endotoxin assays			
Qubit Endotoxin Detection Assay	Qubit Flex	80 assays	Q32891
Qubit Flex Pyrogen-Free Tube Strips	Qubit Flex	120 tube strips	Q32893
Qubit Flex Endotoxin Starter Kit	Qubit Flex	1 kit	Q32894

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