



throughput sensitivity budget

Easy choices for RNA and
DNA quantification

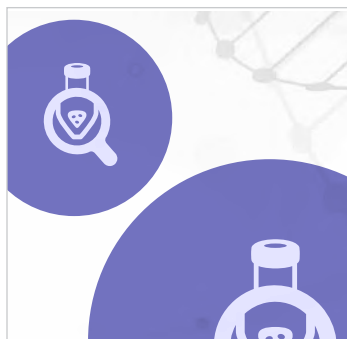
Introduction

DNA quantification 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 proceeding with downstream experiments.

Detecting and quantitating small amounts of nucleic acid is extremely 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 differ in sensitivity, throughput, sample volume required, and information obtained about the sample. Choosing the right technology for your samples, workflow, and throughput can save significant time and money by helping to prevent downstream experimental failures.

Contents



Methods of quantification

UV-Vis technology	4
Fluorescence technology	5

Low throughput

NanoDrop One and One ^c Spectrophotometers	6
Qubit 4 Fluorometer	7



Medium throughput

NanoDrop 8000 Spectrophotometer	8
Qubit Flex Fluorometer	9

High throughput

Multiskan SkyHigh Microplate Spectrophotometer	10
Fluoroskan Microplate Fluorometer	10
Varioskan LUX Multimode Microplate Reader	11



Product listings

12



UV-Vis technology

The 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.

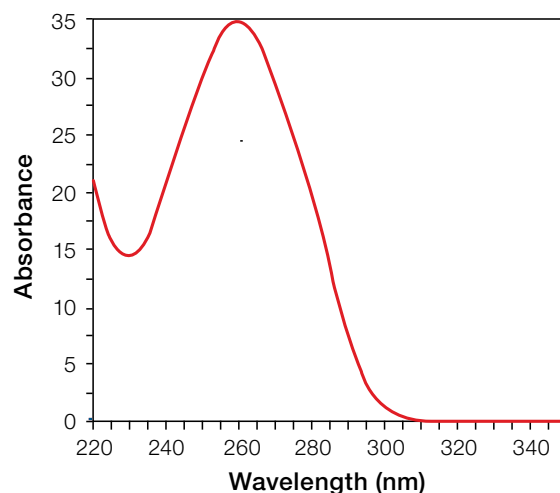


Figure 1. Typical nucleic acid absorbance spectrum.

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 8000	2.5–3,700
	NanoDrop Lite	4–1,500
	Multiskan SkyHigh [*]	1.1–310
	Varioskan LUX ^{**}	0.4–500
Fluorescence	Qubit 4	0.005–2,000
	Qubit Flex	0.005–2,000
	Fluoroskan	0.006–1,000
	Varioskan LUX [†]	0.003–1,000

^{*} 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. Concentrations of nucleic acids are measured using the fluorescence signals of the samples. A calibration curve is generated from standard samples with known concentrations and fit to appropriate regression models. The limit of detection and linear response of the measurements are specific to each assay.

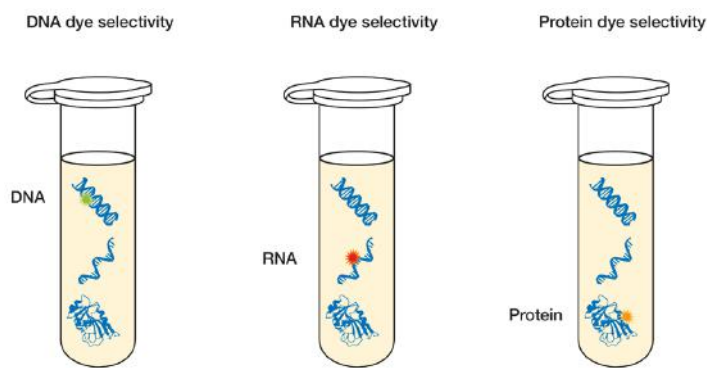


Figure 2. Specificity. Different dyes selectively binding to specific target molecules to be quantified.

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 DNA, dsDNA, ssDNA, 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">• Lacks specificity—does not distinguish 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

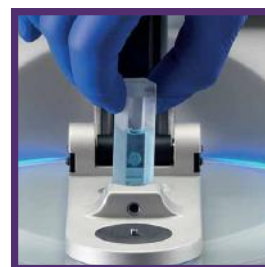
Learn more at thermofisher.com/naq

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 the 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 built into every NanoDrop One instrument.

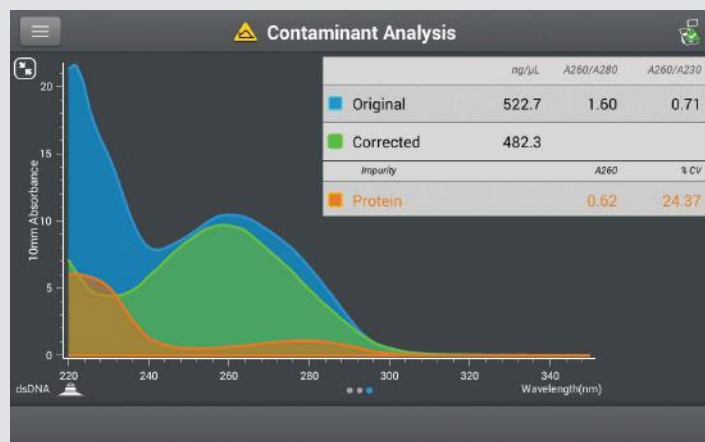
The NanoDrop One^c spectrophotometer adds experimental flexibility and increases the dynamic range. Use cuvettes to measure dilute samples and optical density of bacterial cultures, or to perform kinetic experiments, including with cuvette temperature control and stirring. The cuvette position can be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



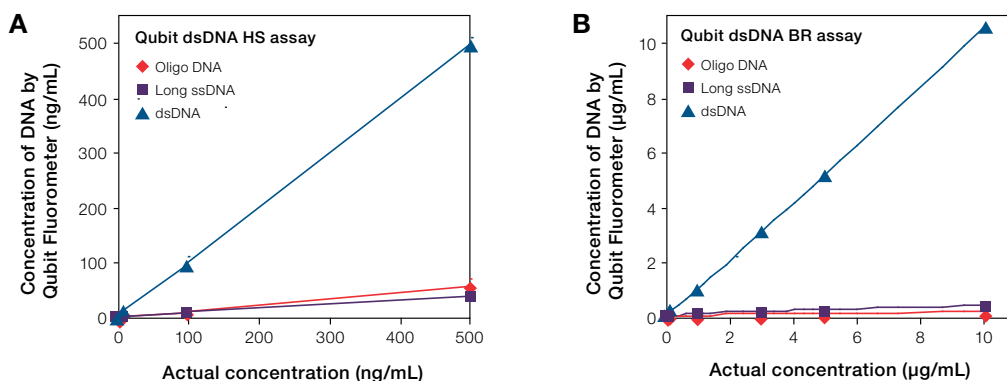
Qubit 4 Fluorometer

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, one sample at a time. 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
- **Plenty of data storage**—stores data for up to 1,000 samples internally
- **Flexible data export**—Wi-Fi, USB drive, or direct connection with a USB cable
- **Portable with small footprint**—small enough to fit in lab coat pocket
- **Optimized reagents and tubes**—Invitrogen™ Qubit™ reagents and tubes work optimally with Qubit fluorometers
- **Intuitive touchscreen**—quickly get to data generation



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 $\mu\text{g/mL}$ in the assay tube using the Qubit dsDNA BR assay, according to kit protocols.

Learn more at thermofisher.com/qubit4

NanoDrop 8000 Spectrophotometer

Measure more samples in less time without sacrificing reliability and ease of use

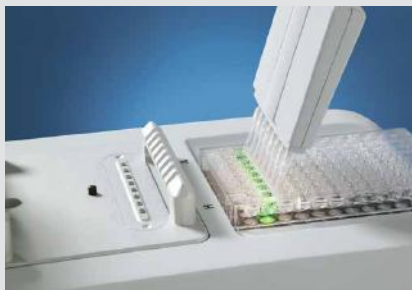
The Thermo Scientific™ NanoDrop™ 8000 spectrophotometer takes full-spectrum UV-Vis absorbance measurements of up to 8 samples simultaneously. Using an 8-channel pipette to dispense samples on a linear array of pedestals, you can easily measure 96 samples in less than 6 minutes.



Key benefits

- Improved productivity with capability of analyzing up to eight 1 μ L samples at one time
- Innovative software to create custom methods and options to design reports and export data
- Increased efficiency with the sample position illuminator, which helps reduce error by keeping track of the samples to be measured
- 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

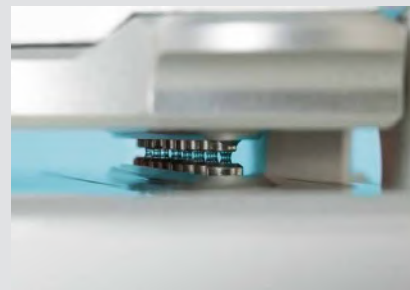
Sample position illuminator



Multi-sample loading



Multi-sample measurement





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, buffer, and sample 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**—Wi-Fi, USB drive, or direct connection with a USB cable
- **Flexible, improved throughput**—measure up to 8 samples per run
- **Portable with small footprint**—easy to move and store on shelves in the lab
- **Optimized reagents and tubes**—Qubit reagents and tubes work optimally with Qubit fluorometers
- **Intuitive touchscreen**—quickly get to data generation

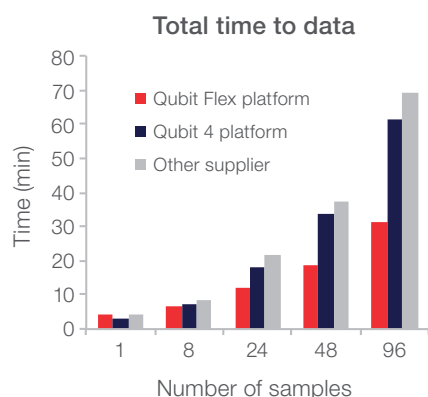


Figure 4. Time trial on 1 to 96 samples shows up to 50% time savings with the Qubit Flex Fluorometer. Time savings began with 8 samples, with more time saved when measuring even more samples.

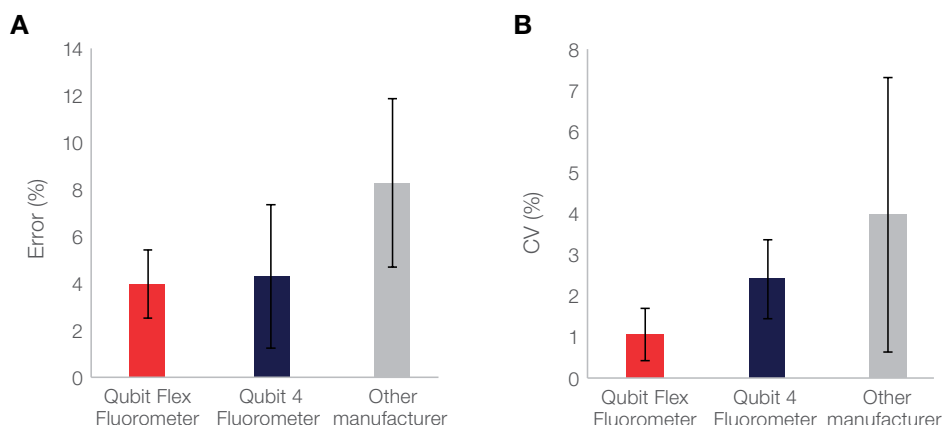


Figure 5. Qubit instruments deliver better accuracy and precision. The Qubit dsDNA BR and 1X dsDNA HS Assay Kits were run with 4 DNA sample concentrations (HS: 0.1, 1, 5, and 10 ng/ μL ; BR: 2, 20, 50, and 100 ng/ μL) in 8 replicates on the Qubit 4 Fluorometer, Qubit Flex Fluorometer, and another manufacturer's 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 microvolume 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



Fluoroskan Microplate Fluorometer

Robust workhorse for sensitive, high-throughput fluorescence detection of nucleic acids

The filter-based Thermo Scientific™ Fluoroskan™ Microplate Fluorometer offers the ability to read plates from 6–384 wells quickly and reliably, with key features so you can be confident in accurate and precise DNA and RNA concentrations.



Key highlights

- Capable of running all the Invitrogen™ Quant-iT™ assays, with the appropriate filters
- Reliable measurement data due to automatic instrument calibration during run
- Dynamic range wider than 6 decades
- Can perform fluorescence measurements with top and bottom reading with high sensitivity
- Operated with Thermo Scientific™ SkanIt™ software for performing even the most complex assays
- Great budget-friendly choice for labs routinely running the same assays

Learn more at thermofisher.com/multiskanskyhigh and thermofisher.com/fluoroskan



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 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

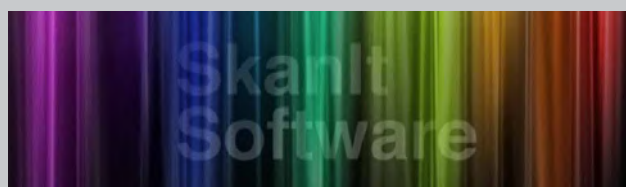
Learn more at thermofisher.com/varioskanlux

Skant Software

Key highlights

- Research Edition is free of charge and can be installed on an unlimited number of computers
- Extremely easy to use, with a logical and intuitive structure
- Drug Discovery Edition is available for 21 CFR Part 11 compliance
- Comprehensive selection of built-in calculations for all assay types is included
- Over 150 ready-made sessions are freely available in the Thermo Scientific™ Skant™ Cloud Library, including all of those needed to execute nucleic acid quantification in all available sample formats, with built-in PLC

Skant Software enables powerful operation and data analysis with all Thermo Scientific™ microplate readers



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 ^c Microvolume UV-Vis Spectrophotometer		ND-ONEC-W or ND-ONEC**
NanoDrop Lite Microvolume UV-Vis Spectrophotometer		ND-LITE
NanoDrop 8000 Spectrophotometer		ND-8000-GL
Multiskan SkyHigh Microplate Spectrophotometer*	thermofisher.com/platereader	A51119500C
Varioskan LUX Multimode Microplate Reader*		VL0000D0
Fluoroskan Microplate Fluorometer*		5200110
Qubit 4 Fluorometer, with WiFi		Q33238
Qubit Flex Fluorometer	thermofisher.com/qubit	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 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 MicroRNA Assay Kit	MicroRNA	Q32880
Qubit RNA IQ Assay Kit†	RNA	Q33221

† Qubit RNA IQ Assay Kit is only compatible with Qubit 4 Fluorometer.

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 dsDNA High-Sensitivity (HS) Assay Kit	dsDNA	Q33120
Quant-iT dsDNA Broad-Range (BR) Assay Kit	dsDNA	Q33130
Quant-iT RNA Assay Kit	RNA	Q33140
Quant-iT RNA Broad-Range (BR) Assay Kit	RNA	Q10213

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

ThermoFisher
SCIENTIFIC

For Research Use Only. Not for use in diagnostic procedures. © 2020 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. AlphaScreen is a trademark of PerkinElmer, Inc.
COL013203 0920