Qubit™ 1X dsDNA BR Assay

Catalog No. Q33265, Q33266

Pub. No. MAN0019617 **Rev.** A.0

Product information

The Qubit™ 1X dsDNA BR (Broad Range) Assay makes DNA quantitation easy and accurate. The kit includes a ready-to-use Working Solution and DNA standards (Table 1). To perform the assay, simply dilute your sample (any volume from 1–20 µL is acceptable) into the 1X working solution provided, then read the fluorescence using a Qubit™ Fluorometer. The assay is highly selective for double-stranded DNA (dsDNA) over RNA (Figure 3, page 6) and is accurate for initial sample concentrations from 200 pg/µL to 4 µg/µL (based on sample volume). The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants such as salts, solvents, detergents, or protein are well tolerated in the assay (Table 2, page 7). In addition to the Qubit™ 1X dsDNA BR Assay described here, we also offer other kits for assaying RNA, protein, and dsDNA at a lower concentration range (Table 3, page 8).

Note: This Qubit™ assay kit can be used with Qubit™ 4 and Qubit™ Flex Fluorometers. If the application is not preloaded on your instrument, download and install the appropriate firmware update file from **thermofisher.com/qubit**.

Table 1. Contents and storage

Material	Amount		Concentration	Storage[1]
	Q33265 (100 assays)	Q33266 (500 assays)		
Qubit™ 1X dsDNA BR Working Solution (Component A)	50 mL	250 mL	1X	• 18–28°C (Room temperature) • Protect from light
Qubit™ 1X dsDNA BR Standard #1 (Component B)	1 mL	5 mL	0 ng/µL in TE buffer	2–8°C
Qubit™ 1X dsDNA BR Standard #2 (Component C)	1 mL	5 mL	100 ng/µL in TE buffer	

[1] When stored as directed, the kit contents are stable for at least 6 months.

Materials required but not provided

- Nuclease-free pipettors and tips
- Qubit™ Assay Tubes (Cat. No. Q32856) or Qubit™ Flex Assay Tube Strips (Cat. No. Q33252)

Critical assay parameters

Assay temperature

The Qubit™ 1X dsDNA BR Assay delivers optimal performance when all solutions are at room temperature (18–28˚C). Temperature fluctuations can influence the accuracy of the assay.

To minimize temperature fluctuations, insert all assay tubes into the Qubit™ Fluorometer only for as much time as it takes for the instrument to measure the fluorescence; the Qubit™ Fluorometers can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading because this warms the solution and results in a different reading.

Incubation time

To allow the Qubit™ 1X dsDNA BR Assay to reach optimal fluorescence, incubate the tubes for 2 minutes after mixing the sample or the standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature when samples are protected from light.

Photostability of Qubit™ reagents

The Qubit™ reagents exhibit high photostability in the Qubit™ Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. However, if the assay tube remains in the Qubit™ Fluorometer for multiple readings, a temporary reduction in fluorescence is observed as the solution increases in temperature. Note that the temperature inside the Qubit™ Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Qubit™ Fluorometer calibration

For each assay, you have the choice to run a new calibration or use the values from the previous calibration. When 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 decide how comfortable you are using the calibration data stored from the last time the instrument was calibrated. Additionally, remember that the fluorescence signal in the tubes containing standards and samples is stable for no longer than 3 hours. See Figure 4 (page 6) for an example of the calibration curve used to generate the quantification results.

Handling and disposal

No data are currently available that address the mutagenicity or toxicity of the Qubit™ 1X dsDNA BR Reagent (the dye in Component A). This reagent is known to bind nucleic acids. Treat the Qubit™ 1X dsDNA BR Working Solution with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Prepare standards and samples

This protocol assumes that you are preparing standards for calibrating the Qubit™ Fluorometer. If you plan to use the last calibration performed on the instrument (see "Qubit™ Fluorometer calibration", page 2), you need fewer tubes (step 1.1) and less working solution (step 1.3).

1.1 Set up the required number of Qubit™ tubes for standards and samples. The Qubit™ 1X dsDNA BR Assay requires 2 standards.

Note: Use only thin-wall, clear, 0.5-mL PCR tubes (Cat. No. Q32856) for the Qubit™ 4 and 8 × 200-µL tube strips (Cat. No. Q33252) for the Qubit™ Flex Fluorometer.

1.2 Label the tube lids.

Note: Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit™ Fluorometer requires the standards to be inserted into the instrument in the right order.

1.3 Add 10 µL of each Qubit™ standard to the appropriate tube.

1.4 Add 1–20 µL of each user sample to the appropriate tube.

Note: If you are adding 1–2 µL of sample, use a P-2 pipette for best results.

1.5 Add the Qubit™ 1X dsDNA BR Working Solution to each tube such that the final volume is 200 µL.

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180–199 µL. Ensure that you have sufficient Qubit™ working solution to accommodate all standards and samples.

Note: To avoid any cross-contamination, we recommend that you remove the total amount of working solution required for your samples and standards from the working solution bottle and then add the required volume to the appropriate tubes instead of pipetting directly from the bottle to each tube.

1.6 Mix each sample vigorously by vortexing for 3–5 seconds.

1.7 Allow all tubes to incubate at room temperature for 2 minutes, then proceed to "Read standards and samples". Follow the procedure appropriate for your instrument:

Read standards and samples

Qubit™ 4 Fluorometer

2.1 On the **Home** screen, touch **DNA**, then select **1X dsDNA BR** as the assay type. Touch **Read Standards** to proceed.

Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 2.4. Otherwise, continue with step 2.2.

2.2 Insert the tube containing Standard #1 into the sample chamber, close the lid, then touch **Read standard**. When the reading is complete (~3 seconds), remove Standard #1.

2.3 Insert the tube containing Standard #2 into the sample chamber, close the lid, then touch **Read standard**. When the reading is complete, remove Standard #2.

Note: The instrument displays the results on the Read Standards screen. For information on interpreting the calibration results, refer to the *Qubit™ 4 Fluorometer User Guide*, available for download at **thermofisher.com/qubit**.

2.4 Touch **Run samples**.

2.5 On the assay screen, select the sample volume and units:

a. Touch the **+** or **–** buttons on the wheel, or anywhere on the wheel itself, to select the **sample volume** added to the assay tube (1–20 µL).

b. From the **unit** dropdown menu, select the units for the output sample concentration.

2.6 Insert a sample tube into the sample chamber, close the lid, then touch **Read tube**. When the reading is complete (~3 seconds), remove the sample tube.

The top value (in large font) is the concentration of the original sample and the bottom value is the dilution concentration. For information on interpreting the sample results, refer to the *Qubit™ 4 Fluorometer User Guide*.

2.7 Repeat step 2.6 until all samples have been read.

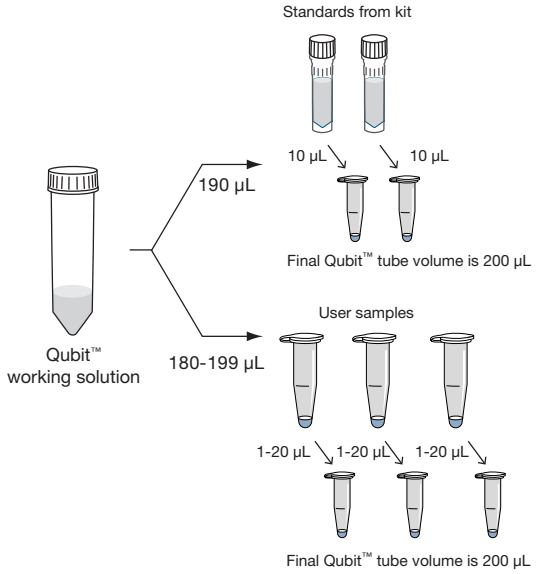

Figure 1. Overview of the Qubit™ 1X dsDNA BR Assay with a Qubit™ 4 Fluorometer.

Qubit™ Flex Fluorometer

3.1 On the **Home** screen, touch **DNA**, then select **1X dsDNA BR** as the assay type. Touch **Read Standards** to proceed.

Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, press **Run samples** and skip to step 3.4. Otherwise, continue with step 3.2.

3.2 Insert the tube strip containing Standard #1 into the sample chamber, close the lid, then touch **Run standards**. When the reading is complete (~3 seconds), remove Standard #1.

3.3 Insert the tube containing Standard #2 into the sample chamber, close the lid, then touch **Run standards**. When the reading is complete, remove Standard #2.

Note: The instrument displays graphical results on the Standards complete screen. For information on interpreting the calibration results, refer to the *Qubit™ Flex Fluorometer User Guide*, available for download at **thermofisher.com/qubit**.

3.4 Press **Next** from the Standards complete screen. When prompted, load the tube strips with your samples as shown in the Insert samples screen. If you have fewer than 8 samples, touch to deselect the tube positions that do not contain a sample.

3.5 Select the units for the output sample concentration, then touch **Next**.

3.6 *(Optional)* Select **More Options** to add the assay kit lot #, tags, or sample IDs. For information on using these options, refer to the *Qubit™ Flex Fluorometer User Guide*.

3.7 In the **Sample volume** screen, enter the sample volume added to the assay tube (1–20 µL). Enter the volume directly in the **Sample volume** text box, use the **+** and **–** buttons, or adjust the **sample volume wheel** to select the sample volume added to the assay tube.

Note: The sample volume used (1–20 µL) changes the assay accuracy range. A different sample volume or assay may be required if the sample concentration is outside of what the assay can accurately quantify.

3.8 Insert a sample tube strip into the sample chamber, close the lid, then touch **Run samples**. When the reading is complete (~3 seconds), remove the sample tube strip.

Standards and sample measurements are displayed on a graph with the results in a list below it.

Touch the graph icon to switch to the results list-only view. The values listed are the concentrations of the original samples. For information on interpreting the sample results, refer to the *Qubit™ Flex Fluorometer User Guide*.

3.9 Select **Add samples** and repeat Step 3.8 to read more samples.

3.10 *(Optional)* Select **Calculators** to access the **Molarity** and **Normalization calculators**. For information on molarity and normalization calculators, refer to the *Qubit™ Flex Fluorometer User Guide*.

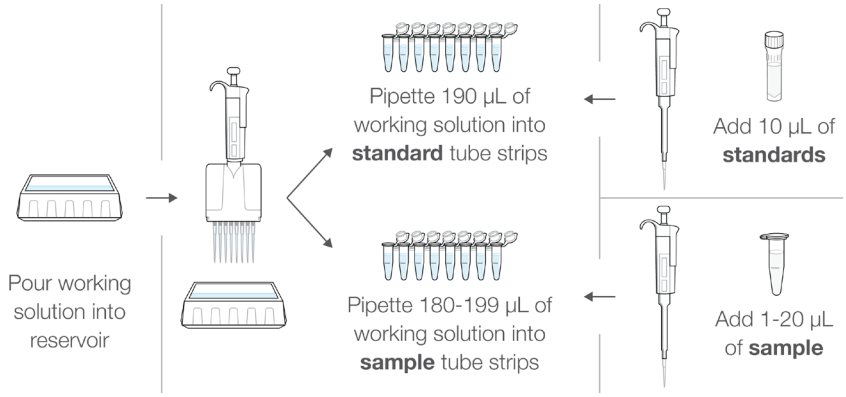

Figure 2. Overview of the Qubit™ 1X dsDNA BR Assay with a Qubit™ Flex Fluorometer.

Appendix

Selectivity of the Qubit™ 1X dsDNA BR Assay

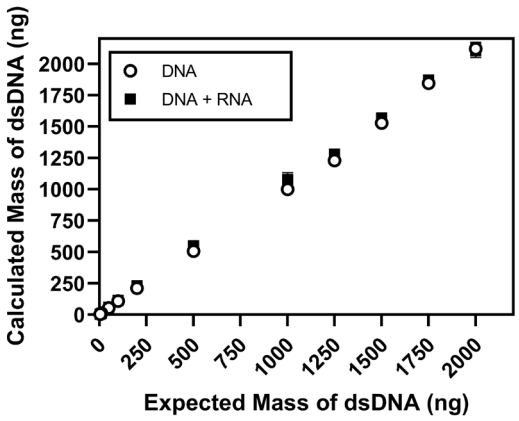

Figure 3. DNA selectivity and sensitivity of the Qubit™ 1X dsDNA BR Assay (Cat. Nos. Q33265, Q33266). Replicate 10-µL samples of λ DNA (Cat. No. Q33263) and a 1:1 mixture of DNA and RNA were assayed with the Qubit™ 1X dsDNA BR Assay. Fluorescence was measured and the calculated mass of the DNA sample was plotted versus expected mass of the DNA sample for the DNA sample alone and for the DNA component in the 1:1 mixture. The coefficient of variance (CV) of replicate DNA determinations was ≤2%.

How the Qubit™ Fluorometer calculates concentration

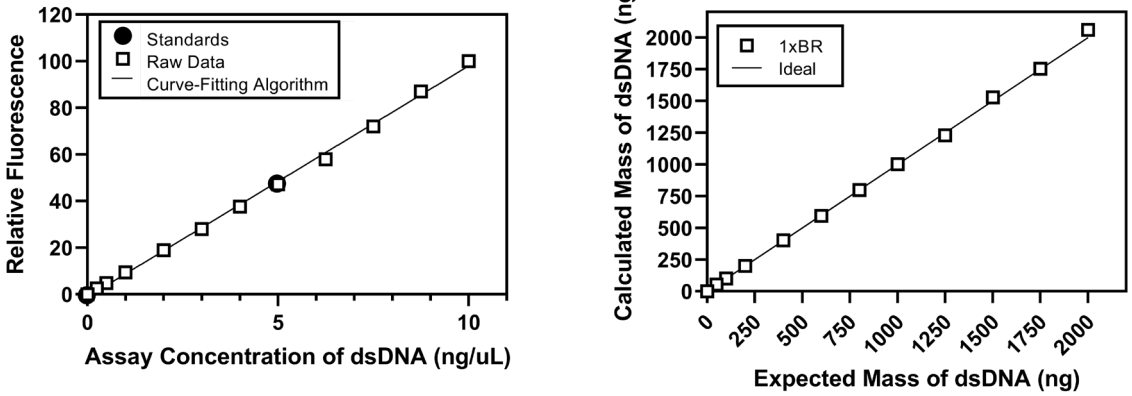

Figure 4. The curve-fitting algorithm used to determine concentration in the Qubit™ 1X dsDNA BR Assay. The Qubit™ Fluorometer generates concentration data based on the relationship between the two standards used in the calibration. The plot on the left shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ 1X dsDNA BR Assay. For reference, the positions of the standards and a set of data points from an actual experiment (right panel) are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation. The application note "Comparison of Quant-iT™ and Qubit™ quantitation assays for accuracy and precision" (available at **thermofisher.com/qubit**) describes in greater detail how the optimized Qubit™ algorithm provides improved accuracy and precision at the low end of the concentration range (<10 ng/mL) over traditional 8-point standard curve and linear regression.

Contaminating substances

A number of common contaminants have been tested with the Qubit™ 1X dsDNA BR Working Solution, and most are well tolerated (Table 2). For untested contaminating substances and in general, the standards should be assayed under the same conditions as the unknowns for highest accuracy. For example, if the experimental samples are in an unusual buffer and if 10 µL volumes of these samples are used, then add 10 µL volumes of the unusual buffer (lacking DNA) to the assays of the standards.

Note: While the contaminant tolerances of the Qubit™ 1X dsDNA BR assay and the Qubit™ dsDNA BR assay are largely similar, they are not identical.

Table 2. Effect of contaminants in the Qubit™ 1X dsDNA BR Assay[1]

Contaminant	Final concentration in the assay	Concentration in 10-µL sample	Concentration in 1-µL sample	Result
Sodium chloride	10 mM	200 mM	2 M	OK
Magnesium chloride	2 mM	40 mM	400 mM	OK
Sodium acetate	10 mM	200 mM	2 M	OK
Ammonium acetate	10 mM	200 mM	2 M	OK
Sodium azide	1 mM	20 mM	200 mM	OK
Urea	5 mM	100 mM	1 M	OK
Guanidine	1 mM	20 mM	200 mM	OK
Ethanol	1%	20%	NA[2]	OK
Phenol	0.1%	2%	20%	OK
Chloroform[3]	0.2%	4%	40%	OK
SDS	0.01%	0.2%	2%	OK
BSA	20 µg/mL	400 µg/mL	4 mg/mL	OK
IgG	10 µg/mL	200 µg/mL	2 mg/mL	OK
ssDNA	3X[4]	3X[4]	3X[4]	OK
RNA	6X[4]	6X[4]	6X[4]	OK
dNTPs[5]	100 µM	2 mM	20 mM	OK

[1] DNA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 10 µL or 1 µL sample volumes are also listed. In all cases, results are given as OK, usually less than 10% perturbation. For best results, add the same amount of contaminant to the standard samples.

[2] User sample would require greater than 100% of listed contaminant.

[3] Immiscible.

[4] 1X indicates a concentration equal to the concentration of dsDNA.

[5] A mixture of dATP, dCTP, dGTP, and dTTP.

NA: Not available.

Qubit™ assay kits compatible with the Qubit™ Fluorometer

A number of fluorescence-based quantification kits are available for use with the Qubit™ Fluorometer. Use Table 3 to choose a kit based on the target molecule and the number of assays you require.

Table 3. Qubit™ assay kits for use with the Qubit™ Fluorometer

Product	Cat. No.	No. of assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	• Core range (high confidence): 0.01 µg/mL to 5 µg/mL† • Extended range (moderate confidence): 5 µg/mL to 10 µg/mL† • Useful for quantitation of genomic and miniprep DNA samples • Accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851 Q33230	100	dsDNA	• Core range (high confidence): 1 ng/mL to 500 ng/mL† • Extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† • Useful for quantitation of PCR products, viral DNA, and samples for subcloning • Accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
	Q32854 Q33231	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	• Core range (high confidence): 5 ng/mL to 1000 ng/mL† • Extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1000 ng/mL to 1200 ng/mL† • Useful for quantitation of oligos, primers, denatured DNA, PCR products • Accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA HS Assay Kit	Q32852	100	RNA	• Core range (high confidence): 25 ng/mL to 500 ng/mL† • Extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1000 ng/mL† • Useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • Accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	• Core range (high confidence): 0.1 µg/mL to 5 µg/mL† • Extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5 µg/mL to 6 µg/mL† • Useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • Accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
	Q10211	500		
Qubit™ RNA XR Assay Kit	Q33233	100	RNA	• Core range: 1 ng/mL to 8 µg/mL† • Useful for quantitation of samples for RT-PCR, qRT-PCR or RNA-SEQ • Accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
	Q33234	500		
Qubit™ microRNA Assay Kit	Q32880	100	RNA	• Core range (high confidence): 5 ng/mL to 500 ng/mL† • Extended ranges (moderate confidence): 2.5 ng/mL to 5 ng/mL and 500 ng/mL to 750 ng/mL† • Useful for quantification of samples for qRT-PCR and sequencing applications • Accurate in the presence of rRNA, large mRNA (>1000 bp), salts, solvents, proteins, and free nucleotides
	Q32881	500		

*Based on an assay volume of 200 µL.

†Concentration ranges refer to the concentration of the sample after dilution in the assay tube.

Table 3 (continued). Qubit™ assay kits for use with the Qubit™ Fluorometer

Product	Cat. No.	No. of assays*	Target	Notes
Qubit™ Protein Assay Kit	Q33211	100	Protein	• Core range (high confidence): 1.25 µg/mL to 25 µg/mL† • Extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† • Little protein-to-protein difference in signal • Accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA • Signal is stable for 3 hours
	Q33212	500		
Qubit™ RNA IQ Assay Kit	Q33221	75	RNA integrity and quality	• Although small in size, the tertiary structure of 5s and tRNA will bind the large RNA dye • Accurate in the presence of salts, protein, solvents and RNA stabilization reagents • Signal is stable for 1 hour • For use with the Qubit™ 4 and Qubit™ Flex Fluorometers; the assay does not work on the original Qubit™, Qubit™ 2.0, or Qubit™ 3 Fluorometers
	Q33222	275		

*Based on an assay volume of 200 µL.
†Concentration ranges refer to the concentration of the sample after dilution in the assay tube.

Ordering information

Cat. No.	Product name	Unit size
Q33265	Qubit™ 1X dsDNA BR Assay Kit, 100 assays	1 kit
Q33266	Qubit™ 1X dsDNA BR Assay Kit, 500 assays	1 kit
Related products		
Q33230	Qubit™ 1X dsDNA HS Assay Kit, 100 assays	1 kit
Q33231	Qubit™ 1X dsDNA HS Assay Kit, 500 assays	1 kit
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng*	1 kit
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng*	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng*	1 kit
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng*	1 kit
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng*	1 kit
Q32852	Qubit™ RNA HS Assay Kit, 100 assays *5–100 ng*	1 kit
Q32855	Qubit™ RNA HS Assay Kit, 500 assays *5–100 ng*	1 kit
Q33221	Qubit™ RNA IQ Assay Kit, 75 assays *for use with the Qubit™ 4 Fluorometer*	1 kit
Q33222	Qubit™ RNA IQ Assay Kit, 275 assays *for use with the Qubit™ 4 Fluorometer*	1 kit
Q32880	Qubit™ microRNA Assay Kit, 100 assays *1–100 ng*	1 kit
Q32881	Qubit™ microRNA Assay Kit, 500 assays *1–100 ng*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg*	1 kit
Q33233	Qubit™ 1X dsDNA HS Assay - Lambda DNA Standard	5 mL
Q33234	Qubit™ 1X dsDNA HS Assay - Calf Thymus DNA Standard	5 mL
Q33235	Qubit™ RNA IQ Assay - RNA Standards	1 set
Q33236	Qubit™ XR Assay - RNA Standard	1 set
Q32856	Qubit™ Assay Tubes	500 tubes
Q33252	Qubit™ Flex Assay Tube Strips	125 tube strips

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited Product Warranty

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Revision history: Pub. No. MAN0019617

Revision	Date	Description
A.0	14 October 2020	New user guide

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