



## sparQ Fast Library Quant Kit

Cat. No. 95197-050      Size: 50 reactions  
95197-500                      500 reactions

Store at -25°C to -15°C  
Protected from light

### Description

Accurate quantification of the number of amplifiable library molecules is one of the most important factors for obtaining high quality read data with next-generation sequencing technologies. The sparQ Fast Library Quant Kit employs qPCR to quantify library molecules that possess the appropriate adapter tag compatible with Illumina® sequencing platforms at each end. This kit was specifically created and optimized for use with the Q real-time PCR instrument, which supports fast cycling protocols with run times of 40 minutes or less.

The 1.25X sparQ Fast Mastermix is premixed with appropriate qPCR primers that target the P5 and P7 Illumina adapter sequences to reduce pipetting steps.

The six stabilized, pre-diluted DNA standards are of known size and concentration for amplification and generation of a standard curve (log pM concentration vs C<sub>q</sub> value), that, through simple trend analysis, allows determination of the concentrations of each unknown diluted library sample.

Analysis may be done either manually by entering C<sub>q</sub> values into the Quantabio companion quantification template or automatically through the Absolute Quantification module of the Q-qPCR software

### Instrument Compatibility

The sparQ Fast Library Quant Kit is optimized for use with the Q real-time PCR cyclers.

Details and specifications of the Q can be found at <https://www.quantabio.com/products-pcr-instrumentation-q>.

### Components

Name	Description	Volume (500 rxn kit)	Volume (50 rxn kit)
Library Dilution Buffer (10X)	10X concentrated buffer containing 0.1M Tris-HCl (pH 8.0), 1 mM EDTA, and stabilizers	2 x 1.5 ml	1 x 1.5 ml
sparQ Fast Mastermix	1.25X sparQ Fast Mastermix containing primers that target the P5 and P7 Illumina adapter sequences	1 x 8 ml	1 x 0.8 ml
sparQ DNA Standard 1	20 pM linear, dsDNA standard in 1X library dilution buffer	1 x 250 µl	1 x 25 µl
sparQ DNA Standard 2	2 pM linear, dsDNA standard in 1X library dilution buffer	1 x 250 µl	1 x 25 µl
sparQ DNA Standard 3	0.2 pM linear, dsDNA standard in 1X library dilution buffer	1 x 250 µl	1 x 25 µl
sparQ DNA Standard 4	0.02 pM linear, dsDNA standard in 1X library dilution buffer	1 x 250 µl	1 x 25 µl
sparQ DNA Standard 5	0.002 pM linear, dsDNA standard in 1X library dilution buffer	1 x 250 µl	1 x 25 µl
sparQ DNA Standard 6	0.0002 pM linear, dsDNA standard in 1X library dilution buffer	1 x 250 µl	1 x 25 µl

### Storage and Stability

Store kit components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt.

For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

## Protocol

### NGS Library Sample Dilutions

- 1) Prepare 1X library dilution buffer with nuclease-free, molecular biology grade water. Add 0.1 ml of 10X Library Dilution Buffer to 0.9 ml of nuclease-free water. Mix by vortexing. Larger or smaller quantities of 1X dilution buffer may be prepared depending on experimental requirements. Each library sample requires approximately 0.4 – 0.6 ml. The 1X dilution buffer is stable at 2 – 8°C for 6 months.
- 2) Prepare dilutions of the NGS libraries to be quantified. Optimal dilutions for qPCR may vary depending on the nature of your NGS sequencing operations. Use the following example showing preparation of 1:10,000 and 1:100,000 dilutions as a general guideline. It is important that one of the library dilutions fall within the linear dynamic range of the supplied sparQ DNA standards.

Prepare a **1:100 dilution** by adding 2 µl of the library sample to a 1.5 ml tube containing 198 µl of 1X Library Dilution Buffer. Mix by vortexing and centrifuge to collect contents.

Prepare the **1:10,000 dilution** by adding 2 µl of the 1:100 dilution sample to a 1.5 ml tube containing 198 µl of 1X Library Dilution Buffer. Mix by vortexing and centrifuge to collect contents.

Prepare the **1:100,000 dilution** by adding 5 µl of the 1:10,000 dilution sample to a 1.5 ml tube containing 45 µl of 1X Library Dilution Buffer. Mix by vortexing and centrifuge to collect contents.

### qPCR Reaction Assembly

The sparQ Fast Mastermix already contains the appropriate qPCR primers and is provided as a 1.25X mix. Addition of diluted library sample or sparQ DNA standard is all that is needed for a complete qPCR reaction.

Reactions can be set up directly in the Q tubes that are supplied with the Q real-time cycler. Each tube is part of a strip of four and provided pre-configured into a loading rack of 48 tubes. All tubes are preloaded with silicone oil that acts as a barrier to prevent evaporation and condensation, thereby removing the need for a heated lid.

We recommend setting up triplicate reactions for each sample or standard. A run on a single Q real-time cycler can accommodate 48 reactions. The Q tubes should be kept in the loading rack while setting up reactions.

The default reaction volume for the sparQ Fast Library Quant kit is 20 µl but 10 µl reactions may also be used to conserve reagents. When adjusting reaction volumes, all components should be scaled proportionally.

Each reaction is set up as follows:

Reaction component	For 20 µl reactions	For 10 µl reactions
sparQ Fast Mastermix (1.25X)	16 µl	8 µl
Diluted library sample or sparQ DNA standard	4 µl	2 µl

- 3) Pipette the appropriate volume of sparQ Fast Mastermix according to the table above into the required number of Q tubes using a repeat pipettor.
- 4) Add the appropriate volume of diluted library sample or sparQ DNA standard as per the table above to appropriate Q tubes held in the Q tube rack.
- 5) To any remaining Q tubes in the rack that do not contain reaction components, add a volume of molecular biology grade water equal to the reaction volume. These water tubes serve as a balance for equal distribution of thermal load around the rotor during the run.

**Note:** Do not mix Q tubes of different reaction volumes in the same run.

- 6) Once all tubes are loaded with reaction or water, use the capping tool to properly fit the caps to the tubes.

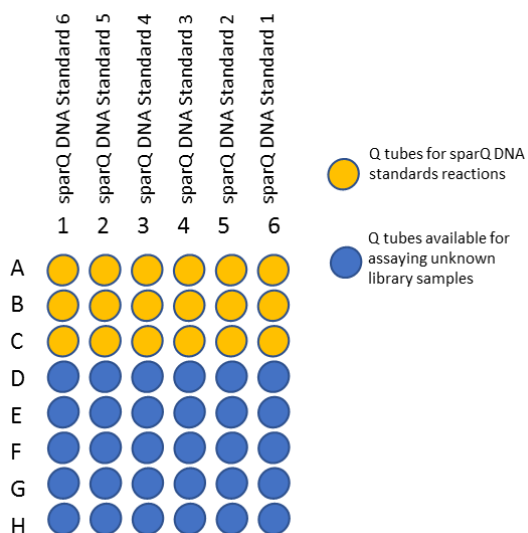
- 7) Load the Q tubes into the Q rotor. The loading rack has engraved numeric and alpha numeric identifiers next to each tube. Each strip of four tubes also has one tube marked with a tab. Use the tube identifier and tab positions from the loading rack to load Q tubes into the corresponding positions in the Q rotor.
- 8) After loading all the rotor positions, place the tube clamp on the top of the rotor. This serves as a safeguard against any of the tubes or caps coming out of the rotor during a run.

**Note:** It is not necessary to vortex tubes prior to loading into the Q rotor. The initial centrifugation step of a run spins samples through the silicone oil overlay and provides adequate mixing of reaction components.

## Software Setup and qPCR Amplification

- 9) Two template files are available for download from the Quantabio website at [www.quantabio.com/sparq-fast-quant](http://www.quantabio.com/sparq-fast-quant).  
Download the file named [sparQ Fast Library Quant Template 20ul.QTemplate](#) if reactions are 20 µl volume.  
Download the file named [sparQ Fast Library Quant Template 10ul.QTemplate](#) if reactions are 10 µl volume.
- 10) Transfer the template file to the Q software template library of the PC located along the path: **My Documents > Quantabio > Q-qPCR > Templates**.  
  
**Note:** Template files must be in the appropriate folder location and can only be opened from within the Q-qPCR software.
- 11) Double click on the icon to open the Q-qPCR software. Select **[New]** then select **[Run from Template]**.
- 12) Choose the appropriate template file that was downloaded and transferred to the Q software template library in steps 9 and 10. This will load the appropriate parameters for the assay and run profile.

**Note:** The template files are preconfigured with information about the six sparQ DNA standards included as triplicate reactions in the run. These are intended for the first three Q tubes in each column of the Q rack as mapped below and should be handled from lowest to highest concentration to avoid cross contamination. This setup allows room for triplicate reactions of 10 unknown samples per run. The optimized cycling conditions are shown on the right. If further flexibility is needed with sample locations, the number or replicates, or cycling parameters please consult the **sparQ Fast Library Quant technical guide** available for download at [www.quantabio.com/sparq-fast-quant](http://www.quantabio.com/sparq-fast-quant).



Activation:	95°C; 2 min
35 Cycles:	95°C; 5 sec
	65°C; 25 sec
Melt:	72°C to 95°C at 0.3°C/sec

- 13) Select the [Samples] tab and enter the sample names in the appropriate rows of the Name column of the sample editor. The Q tube rack positions of the samples can be displayed either numerically (1, 2, 3 ...) or alpha numeric (A1, B1, C1 ...) by clicking the icon located at the top of the sample editor screen.



- 14) To start the run, click on the Q instrument icon and then select **[Start Run]**. When prompted, save the run file to the computer desktop or other designated data folder. Once named and saved, the run will commence and will complete in approximately 40 minutes.
- 15) Upon completion, save the file again. Analysis can be performed in the Q-qPCR software using the Cycling, Melt, or Absolute Quantification tabs or the results can be output into an Excel Workbook by choosing **[Save As] > [Excel Workbook]**.

## Quality Control

qPCR of the DNA Standards are assayed in triplicate reactions with sparQ Fast Mastermix on the Q according to manufacturer's recommended cycling conditions and must generate a standard curve with  $R^2 \geq 0.995$  and a PCR efficiency  $\geq 90\%$ . Dissociation curve analysis must present as a single melt peak with the expected  $T_m$ .

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## Related Products

### sparQ DNA Frag & Library Prep Kit

Cat. No.	95194-024	Size:	24 reactions
	95194-096		96 reactions

### sparQ DNA Library Prep Kit

Cat. No.	95191-024	Size:	24 reactions
	95191-096		96 reactions

### sparQ Adapter Barcodes Set A

Cat. No.	95193-A96	Size:	96 reactions
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### sparQ Adapter Barcodes Set B

Cat. No.	95193-B96	Size:	96 reactions
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### sparQ HiFi PCR Master Mix

Cat. No.	95192-050	Size:	50 reactions
	95192-250		250 reactions

The sparQ HiFi PCR Master Mix is a high efficiency, high-fidelity, and low bias PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing. The included primer mix allows amplification of DNA libraries flanked by adapters containing the P5 and P7 Illumina® flow cell sequences.

### sparQ PureMag Beads

Cat. No.	95196-005	Size:	5 ml
	95196-060		60 ml
	95196-450		450 ml

### Q Tubes & Caps (20 racks/box)

Cat. No.	95910-20	Size:	20 racks of 48 tubes/box
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