



sparQ Universal Library Quant Kit

Cat. No: 95210-100 Size: 100 x 20 μ l reactions
95210-500 500 x 20 μ l 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 Universal Library Quant Kit employs qPCR to quantify library molecules that possess the appropriate Illumina[®] NGS library adapter tag at each end. Reagents are optimized to support fast cycling protocols with run times under 45 minutes.

The 1.25x sparQ Universal Fast Mastermix is configured 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. Cq value), that, through simple trend analysis, allows determination of the concentrations of each unknown diluted library sample.

Instrument Compatibility

The sparQ Universal Library Quant Kit is compatible with all real-time PCR instruments capable of reading fluorescent emission in the Green channel (~560-520 nm). It contains a ROX[®] reference dye that is compatible with qPCR systems that use optimized excitation and emission filter sets for each dye-detection channel ("low ROX" instruments), as well as qPCR systems that do not require a passive reference dye ("no ROX" instruments). For qPCR systems that use a fixed excitation wavelength for all dye detection channels ("high ROX" instruments), additional ROX Reference Dye is provided in a separate vial, which can be added to the Mastermix.

Appendix A gives a list of the most prevalent real time cyclers and ROX requirements. Please refer to your qPCR system's instructions for proper reference dye settings.

Components

Name	Description	Volume (100 rxn kit)	Volume (500 rxn kit)
sparQ Universal Fast Mastermix	1.25x sparQ Universal Fast Mastermix containing primers that target the P5 and P7 Illumina adaptor sequences	1 x 1.6 ml	1 x 8 ml
sparQ DNA Standard 1	20 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 μ l	1 x 250 μ l
sparQ DNA Standard 2	2 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 μ l	1 x 250 μ l
sparQ DNA Standard 3	0.2 pM linear, dsDNA standard in 1x library dilution buffer	1 x 25 μ l	1 x 250 μ l
sparQ DNA Standard 4	0.02 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 μ l	1 x 250 μ l
sparQ DNA Standard 5	0.002 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 μ l	1 x 250 μ l
sparQ DNA Standard 6	0.0002 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 μ l	1 x 250 μ l
High ROX Reference Dye	High ROX reference dye for use with "High ROX" instruments.	1 x 25 μ l	1 x 250 μ l
Library Dilution Buffer (10x)	10x concentrated buffer containing 0.1M Tris-HCl (pH 8.0), 1 mM EDTA, and stabilizers	1 x 1.5 ml	2 x 1.5 ml

Storage and Stability

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

Protocol

Preparation of 1x library dilution buffer and 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 Library Dilution Buffer may be prepared depending on experimental requirements. Each library sample requires approximately 0.4 - 0.6 ml. The 1x Library 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.

Preparation of the sparQ Universal Fast Mastermix for users of “high ROX” instruments

The sparQ Universal Fast Mastermix is a 1.25x mix that contains the appropriate qPCR primers and a low level of ROX passive reference dye ready for use with “low ROX” and “no ROX” instruments.

Users of “High ROX” instruments must spike in additional ROX upon first opening the mastermix. This is done by taking 4.5 µl from the separate vial of ROX provided with the kit and adding it directly to the 1.6 ml of sparQ Universal Fast Mastermix. Thoroughly mix by gently vortexing for 30 seconds. Continue through the rest of the protocol.

qPCR Reaction Assembly

Addition of diluted library sample or sparQ DNA standard is all that is needed for a complete qPCR reaction.

The default reaction volume for the sparQ Universal Library Quant kit is 20 µl.

We recommend setting up triplicate reactions for each sample or standard.

- 3) Set up each reaction on ice as follows:

sparQ Universal Fast Mastermix	16 µl
Diluted library sample or sparQ DNA standard	4 µl

(Smaller reaction volumes can be used by scaling components proportionally)

Dispense 16 µl of sparQ Universal Fast Mastermix into the required number of plate wells.

Add 4 µl of diluted library sample or sparQ DNA standard to appropriate wells.

Seal the plate, briefly vortex to mix reactions, and briefly centrifuge to collect contents to the bottom of each well.



Appendix A

Real-Time PCR Systems that do not require ROX ("no ROX")	Quantabio Q, Bio-Rad CFX96™, CFX384™, Opticon™, MiniOpticon™, Chromo4™, iQ™5*, MyiQ™*, iCycler iQ®* (*requires persistent well factors); Cepheid Smart Cyclyer®; Qiagen Rotor-Gene®; Illumina® Eco™; Eppendorf Mastercycler® ep realplex; Roche LightCycler® 480, 96
Real-Time PCR Systems that require low concentration ROX ("low ROX")	Applied Biosystems 7500, 7500 Fast, ViiA™ 7, QuantStudio™; Agilent/Stratagene MX4000™, MX3005P™, MX3000P™
Real-Time PCR Systems that require high concentration ROX ("high ROX")	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™

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