sparQ Universal Library Quant Kit

Cat. No: 95210-100 Size: 100 x 20 µl reactions
95210-500 500 x 20 µl 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 adapter 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 µl	1 x 250 µl
sparQ DNA Standard 2	2 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 µl	1 x 250 µl
sparQ DNA Standard 3	0.2 pM linear, dsDNA standard in 1x library dilution buffer	1 x 25 µl	1 x 250 µl
sparQ DNA Standard 4	0.02 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 µl	1 x 250 µl
sparQ DNA Standard 5	0.002 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 µl	1 x 250 µl
sparQ DNA Standard 6	0.0002 pM linear, dsDNA standard in 1x Library Dilution Buffer	1 x 25 µl	1 x 250 µl
High ROX Reference Dye	High ROX reference dye for use with "High ROX" instruments.	1 x 25 µl	1 x 25 µl
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	5 x 1.5 ml

- Additional volume of Library Dilution Buffer (10x) can be purchased separately.

Part Number	Description	Kit Size
95210-15B	sparQ Library Dilution Buffer	15 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.

High ROX" instruments require spike in of additional ROX upon first opening the mastermix. For the 100 rxn kit, add 5 µl of High ROX Reference Dye directly to the 2 ml tube of sparQ Universal Fast Mastermix. For the 500 rxn kit, spin down High ROX Reference Dye, then add 25 µl to the bottle 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.

qPCR Amplification

4) Program your real-time qPCR instrument

Define wells containing the sparQ DNA standards as "standard" with the appropriate concentrations given in the [Components] table above (20 pM – 0.0002 pM).

Define diluted NGS library samples as "unknown".

Group replicate reactions to allow the qPCR data analysis program to calculate average Cq and concentrations based on the sparQ DNA standards.

5) Incubate the reaction plate in a real-time qPCR system as follows:

Initial denaturation:		95°C, 2 min
PCR cycling (35 cycles)	Denaturation:	95°C, 5 sec
	Annealing / Extension:	65°C, 25 sec

Dissociation (melt) curve is optional – refer to qPCR instrument manual.

Data Analysis

The reaction plate should be defined, or annotated, using the stated concentrations for each DNA standard. Since equal volumes of each DNA standard and library dilution sample were used in each qPCR, a simple trend analysis of the standard curve (log pM concentration vs Cq value) linear regression can be used to calculate the pM concentration for each library sample. This should be carried out automatically using the absolute (or standard curve) quantification algorithm provided with your qPCR detection system. There is no need to determine the template copy number or template concentration of each qPCR. Please consult your qPCR system documentation.

The Illumina DNA standards and primer mix generate a 426-bp amplicon (48.8% GC). A common problem with some NGS library quantification protocols is the use of DNA standards that are overly concentrated and generate qPCR data that are outside of the linear dynamic range for most qPCR instruments. Improper baseline settings result in compressions between the highest concentrated DNA standards, which in turn give rise to inflated PCR efficiencies and inaccurate library quantification results. The sparQ DNA standards supplied with the sparQ Universal Library Quant Kit have been selected to avoid these artifacts and produce absolute quantification standard curves with exceptionally high linear regression correlation coefficients.

Size-adjusted concentration (pM) for each library dilution sample is obtained as follows:
average concentration (pM) x (426 / average fragment length)

The size-adjusted concentration of the original library sample is obtained by multiplying the value above by the dilution factor used for the qPCR (either 10,000 or 100,000). It is important to only use Cq data from library dilution samples that fall within the linear dynamic range of the DNA standards. Use the size-adjusted concentration of the original library to determine the appropriate dilution to use for loading the flow cell for the bridge amplification. The optimal loading concentration may vary depending on sample type, library construction method, and Illumina instrumentation.

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 Cycler®; 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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