JetSeq™ Library Quantification Lo-ROX Kit

Shipping: On dry/blue ice

Catalog numbers:

BIO-68029: 500 x 20 µL reactions

Batch No.: See vial

Concentration: 2x

Store at –20°C

BIOLINE

A Meridian Life Science® Company

Storage and stability:
The JetSeq Library Quantification Lo-ROX Kit is shipped on dry/blue ice. On arrival store at -20°C for optimum stability. Excessive freeze/thaw cycles are not recommended.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:
Please refer to the material safety data sheet for further information.

Notes:
Research use only

Description

The JetSeq™ Library Quantification Lo-ROX Kit contains sufficient reagents for the production of at least twenty DNA standard curves. This will allow the quantification of up to 18 DNA libraries on separate 96 or 384 well plates and up to a maximum of 62 libraries on six 96 well plates or 76 libraries on two 384 well plates.

The JetSeq Library Quantification Lo-ROX Kit is optimized for fast and robust quantification of Illumina-based next generation sequencing (NGS) libraries. It consists of JetSeq FAST Lo-ROX Mix (a SYBR®-based qPCR mix), six pre-diluted DNA standards (ranging from 10 pM to 100 aM to minimize pipetting errors), JetSeq Primer Mix (to ensure reproducible and precise qPCR results) and a JetSeq Dilution Buffer (optimized for dilution of NGS library samples).

Components

Reagent	500 x 20 µL reactions
JetSeq Primer Mix	2 x 1.25 mL
JetSeq FAST Lo-ROX Mix	5 x 1 mL
JetSeq Dilution Buffer	5 x 5 mL
DNA Standard 1 (10 pM)	1 x 300 µL
DNA Standard 2 (1 pM)	1 x 300 µL
DNA Standard 3 (100 fM)	1 x 300 µL
DNA Standard 4 (10 fM)	1 x 300 µL
DNA Standard 5 (1 fM)	1 x 300 µL
DNA Standard 6 (100 aM)	1 x 300 µL

Kit compatibility

The JetSeq Library Quantification Lo-ROX Kit can be used on several types of qPCR instruments. When used on ABI 7500, 7500 Fast, ViiA7™, Agilent Mx3000P™, Mx30005P™ or Mx4000™, the user has the choice of analyzing the qPCR data with the passive reference signal either on or off. If your qPCR instrument has the capability of using ROX and you wish to use this option, then this option must be selected by the user in the software.
The JetSeq Library Quantification Lo-ROX Kit can also be used on instruments that do not require the use of ROX (5-carboxy-X-rhodamine, single isomer), such as the BMS Mic, BioRad® Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384, iQ5™, Cepheid® SmartCycler™, Qiagen (Corbett) Rotor -Gene™ 3000, 6000 & Q, Analytik Jena qTower2, Eppendorf Mastercycler ep Realplex, ep Realplex 2S, Roche LightCycler® 480, LightCycler® Nano, Techne Quantica®, PrimeQ, PCRmax Eco™, Takara Thermal Cycler Dice® TP800.

General considerations

The 342 bp fragment used as a standard in this protocol (labelled DNA Standards 1-6) consists of a linear DNA fragment flanked by additional stabilizing DNA. The DNA Standards should only be used in conjunction with the other reagents in this kit for the quantification of DNA libraries prior to use in Illumina-based next generation sequencing.
To help prevent any carry-over DNA contamination, we recommend that separate areas maintained for library preparation, reaction set-up, PCR amplification and any post-PCR gel analysis.

It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area. It is highly recommended that no template controls (NTC) are included in each assay to detect contamination introduced during reaction set-up. NTC reaction should give Ct values that are at least 3.5 cycles later than the average Ct value for Standard 6.

Recommended Protocol

Preparation of the library sample dilutions:
We recommend preparing a 1:10,000 diluted sample of the library using the JetSeq Dilution Buffer. In order to improve the accuracy of the quantification, we suggest including additional dilution samples of each library (such as 1:100,000 and/or 1:1,000,000). Using two or more different dilutions of the library will ensure that at least one dilution falls within the dynamic range of the standard curve generated. This could be especially useful when the library concentration is high.

Reaction set-up:
Prepare a qPCR mastermix. The volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly. In order to improve the accuracy and reproducibility of the quantification we recommend assaying each standard and library sample in triplicate. In addition, setting-up the reactions using a mastermix will reduce pipetting errors and will increase reproducibility.

Reagent	Volume
2x JetSeq Library Quantification Lo-ROX Mix	10 µL
Primer Mix	5 µL
Diluted library or DNA standard (1-6)	5 µL
Total volume	**20 µL final volume**

Recommended cycling conditions:
The recommended settings for a typical qPCR machine are listed over. Optimization of these conditions, may be required for slower qPCR machines.

Website: www.bioline.com/ email: info@bioline.com

Cycles	Temp.	Time	Notes
1	95°C	2 min	Polymerase activation
35	95°C 60°C	5 s 45 s*	Denaturation Annealing/extension (acquire at end of step)
Melt curve			Acquiring in green

*If the average library fragment size is larger than 500 bp, then increase annealing/ extension time to 60 s.

Optional analysis: After the reaction has reached completion, refer to the instrument instructions for the option of melt-profile analysis.

Analysis and Quantification

Generation of a standard curve from the standard samples:
Correlate the concentration values of the different standard dilutions against their respective Ct (averaged from each triplicate) using data generated by qPCR. This data can be used to calculate the efficiency of the reaction by generating a standard curve. This can be obtained by plotting the average Ct value against log (concentration). Many qPCR machines generate this type of curve using the software provided by the manufacturer. The efficiency of the reaction is calculated using the following formula:

$$\textit{Efficiency}\ (\%) = (10^{\left(\frac{-1}{a}\right)} - 1) \times 100$$

Where a is the slope of the standard curve. The software provided with many qPCR machines often provides routines to display this value.
Check that the reaction efficiency is between 90-110% for the DNA standards. If the value falls outside of these parameters, please refer to "Troubleshooting" below.

Quantification of the library concentration:
From the Ct values obtained for the library sample dilutions, the library concentration is calculated using the standard curve following the formula, *where the concentration of the library (*L) *in picomolar is given by:*

$$\text{L (pM)} = 10^{\frac{Ct-b}{a}} \times \frac{342}{\textit{average fragment length}} \times \textit{dilution factor}$$

Where a and b represent the standard curve slope and y-intercept respectively. The formula above uses a fragment length for the standards of 342 bp.

Sequence of the primers:

- F: 5'-AAT GAT ACG GCG ACC ACC GA-3'
-R: 5'-CAA GCA GAA GAC GGC ATA CGA-3'

Troubleshooting

Problem	Possible cause	Recommendation
Variability between replicates	Error in reaction assembly	Prepare large volume mastermix, vortex thoroughly and aliquot into reaction plate.
	Air bubbles in reaction mix	Centrifuge reaction samples/plates prior to running on a qPCR instrument.
Non-amplification traces in the diluted library samples	Non-compatible library	Confirm that the qPCR primer sequences provided in this kit are compatible with the adaptor sequences used to construct the libraries to be quantified.
	Error in instrument setup	Check that the acquisition setting is correct during cycling.
Library is outside of the dynamic range of the assay	High DNA concentration in library	Increase the dilution factor.
	Low DNA concentration in library	Reduce the dilution factor.
Extra peak in melt curve(s)	Internal contamination with other DNA libraries	Always set up a no template control reaction (NTC). NTC reactions should give Ct values that are at least 3.5 cycles later than the average Ct values for Standard 6.

Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact your local distributor or our Technical Support with details of reaction set-up, cycling conditions and relevant data.

Email: tech@bioline.com

Associated Products

Product Name	Cat No
JetSeq DNA Library Preparation Kit	BIO-68025
JetSeq Flex DNA Library Preparation Kit	BIO-68026
JetSeq ER and Ligation DNA Library Preparation Kit	BIO-68027
Mic - Personal qPCR Cycler	BMS-MIC-2

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