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EliZyme™ Library Quantification Kit

Intended use:

For research use only. Not for use in diagnostic procedures.

Storage:

Upon arrival store components at -20 °C. Avoid prolonged exposure to light. When stored under these conditions, the kit will retain full activity until the expiration date indicated on the kit label.

Introduction

Accurate quantification of library plays a crucial role in next-generation sequencing. When pooling libraries, any deviation of concentration between samples can result in variations in the number of sequenced reads. Low library concentration may result in low cluster density, whereas high library concentration may result in higher cluster density, which can lead to poor cluster resolution. Both cases result in poor data quality.

Product description

EliZyme™ Library Quantification Kit is based on qPCR method. **EliZyme™ Library Quantification Kit** for Illumina® contains all the reagents required for successful library quantification. Library quantification is performed by amplification of pre-diluted DNA standards and diluted library samples. Primers recognize the adapter sequences used for Illumina® next-generation sequencing. The average C_t value of each DNA standard is plotted against concentration in pM (in log scale) generating a standard curve used for calculating library concentration.

Content

	Ref. no.	Size	Package
EliZyme™ Library Quantification Kit	EZ4815	500/1000 rxns (10 µl or 20 µl reactions)	2x EliZyme™ qPCR Mix (5 ml) Primer Mix (1 ml) DNA Standards 1-5 (Ref. no. DNAS, 80 µl) ROX Additive (100 µl)

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Content	Concentration
DNA Standard 1	20 pM
DNA Standard 2	2 pM
DNA Standard 3	0.2 pM
DNA Standard 4	0.02 pM
DNA Standard 5	0.002 pM
Primer Mix	10x Primer Premix
2x EliZyme™ qPCR Mix	2x Reaction
ROX Additive	50 µM

Reaction Setup

Before the usage, all reagents must be completely thawed, briefly mixed on vortex and shortly spun. Pay attention to complete thawing of DNA standards. Unthawed DNA standards may cause differences in calibration curve, thus leading to inaccurate determination of library concentration.

Different levels of ROX passive reference are required for different real-time PCR cyclers. The 50µM ROX Additive is added directly to the 5 ml tube of 2x EliZyme™ qPCR Mix. After adding the ROX, the reagent may be used right away or stored at -20 °C for future use. Check your instrument for adding passive reference dye. For adding the correct amount of ROX use the following instructions.

For NO-ROX cyclers

Reagents for Master Mix		20 µl rxn	10 µl rxn
2x EliZyme™ qPCR Mix	5.0 ml	Master Mix 12 µl	Master Mix 6 µl
Primer Mix	1.0 ml	+ PCR-grade water 4 µl	+ Sample 4 µl*
		+ Sample 4 µl*	

*DNA Standards, diluted library or PCR-grade water

For Low-ROX cyclers

Reagents for Master Mix		20 µl rxn	10 µl rxn
2x EliZyme™ qPCR Mix	5.0 ml	Master Mix 12 µl	Master Mix 6 µl
Primer Mix	1.0 ml	+ PCR-grade water 4 µl	+ Sample 4 µl*
ROX Additive	10.0 µl	+ Sample 4 µl*	

*DNA Standards, diluted library or PCR-grade water

For High-ROX cyclers

Reagents for Master Mix		20 µl rxn	10 µl rxn
2x EliZyme™ qPCR Mix	5.0 ml	Master Mix 12 µl	Master Mix 6 µl
Primer Mix	1.0 ml	+ PCR-grade water 4 µl	+ Sample 4 µl*
ROX Additive	100.0 µl	+ Sample 4 µl*	

*DNA Standards, diluted library or PCR-grade water

Sample preparation

Prepare the appropriate library dilution. Diluted library must be in range of calibration curve. Avoid extremely large dilutions. Instead of one large dilution perform 2-fold dilution (e.g. perform two 1/100 dilutions instead of 1/10,000 dilution). For each quantification use freshly diluted library.

Triplicates of qPCRs are recommended for DNA Standards, library samples and controls.
Use PCR-grade water as a negative control for each run. C_t scores of negative controls should be at least 3 cycles later than the average C_t scores for Standard 5.

PCR protocol

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	95 °C	10 s	35
Annealing/Extension	60 °C	30 s*	
Melt curve analysis**	65 °C – 95 °C		

*For amplicons longer than 700 bp increase to 90 s.
**Melt curve analysis is useful to determine the presence of adapter-dimers in library or to determine contamination.

Data analysis

To determine the concentration of the library use the standard curve. Ensure that the standard curve has an R-squared value ≥ 0.99, amplification efficiency between 80 – 120 % and the average ΔC_t value is 3.2 – 4.2. If the standard curve does not meet the listed criteria, the library quantification may not be accurate.
If the average C_t score of a diluted library is lower than that of DNA Standard 1 or higher than that of DNA Standard 5, exclude it. Use appropriate dilution of the library. The concentration should fall within the standard curve.

When calculating library concentration, use the average C_t value of particular dilution. If only one library dilution falls within the standard curve, use that concentration value.
Concentration has to be adjusted to the average target length for the library. To calculate adjusted concentration multiply averaged measured concentration with the following fraction:

Length of DNA Standard (447 bp)/Average fragment length of library in bp

Multiply adjusted concentration with the dilution factor of each used library. Determine the concentration to be used for pooling libraries or cluster amplification.

Manufacturer:

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REF	Catalog number		Upper limit of temperature
LOT	Batch code		Manufacturer
	Use by (last day of month)	Σ	Contains sufficient "N" tests