



## NGS cfDNA Library Quantification Kit for Illumina

**Project number:** N665917

**Storage condition:** -20 °C, 12 months, if need to be used frequently, can be stored in 2-8°C, try to avoid repeated freezing and thawing.

### Product content

Component	N665917-1ml	N665917-5ml
2×SYBR qPCR MasterMix	1ml	5 x 1ml
qPCR Primer Mix	100µl	500µl
DNA Standard A	100µl	500µl
DNA Standard B	100µl	500µl
DNA Standard C	100µl	500µl
DNA Standard D	100µl	500µl
DNA Standard E	100µl	500µl
50 x High ROX	40µl	200µl

### Product Introduction

This is a dye-based (SYBR Green I) qPCR NGS library quantification kit for cfDNA, which provides the reaction mixture, DNA primer mixture, standards, and sample dilutions required for the qPCR process, making it a complete reagent system that is easy and convenient to use. The fluorescent dye SYBR Green I contained in the reaction mixture binds to all double-stranded DNA. The kit uses a new chemically modified high-efficiency hot-start polymerase, the activation of the enzyme needs to be incubated at 95 °C for 10 min. the product is highly specific, high amplification efficiency, the length of the standard in the kit (about 270bp) is comparable to the average length of the cfDNA NGS libraries (250-300bp), which is able to quickly and accurately quantitate the concentration of the constructed cfDNA libraries. quantification.

ROX dye is used to correct the fluorescence signal error generated between wells of a quantitative PCR instrument, and is generally used in Real Time PCR amplifiers from ABI, Stratagene, and other companies. The excitation optics vary from instrument to instrument, so the concentration of ROX dye must be matched to the corresponding fluorescence quantitative PCR instrument.

Instruments that do not require ROX calibration: Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, etc.

Instruments requiring Low ROX calibration: ABI Prism7500/7500 Fast, QuantStudio®3 System, QuantStudio®5 System, QuantStudio®6 Flex System, QuantStudio®7 Flex System, ViiA7 System, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, and others.

Instruments requiring High ROX calibration: ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, etc.

NOTE: High Rox and Low Rox are formulated as described in Method of Use 2.

### Applicable scope

This product is designed for the absolute quantification of the concentration of Illumina platform second generation sequencing libraries. The end of the library contains Illumina P5 and P7 microarray binding sequences, the length of which does not exceed 1kb, and the concentration is not less than 0.02pM can be used for quantitative experiments. The qPCR Primer Mix provided in the kit

contains the following two primer sequences:

Primer 1: 5'-AAT GAT ACG GCG ACC ACC GA-3' Primer 2: 5'-CAA GCA GAA GAC GGC ATA CGA-3'

The primer sequence can be used in advance to confirm whether the library can be amplified by that primer pair.

## Usage

### Amplification template preparation

The library samples to be detected were diluted with TE (10 mM Tris-Cl, pH 8.0, 1 mM EDTA), and the concentration after dilution was as close as possible to the range of 0.01-60 pM. 4° C on ice was set aside.

### qPCR reaction system preparation

The desired cryopreservation reagent is pre-melted completely and mixed by inverting several times before preparation, then centrifuged briefly and set aside.

The base reaction system for 20  $\mu$ l was as follows:

reagents	20 $\mu$ l reaction system
2 $\times$ SYBR qPCR MasterMix	10 $\mu$ l
qPCR Primer Mix	0.8 $\mu$ l
Template	4 $\mu$ l
ddH <sub>2</sub> O	5.2 $\mu$ l

Description: High Rox model: 1  $\mu$ l High Rox per 50  $\mu$ l of reaction system; Low Rox model: 1  $\mu$ l High Rox per 500  $\mu$ l of reaction system.

Prepare a sufficient amount of reaction system mixture according to the need, mix well and add to the reaction wells in a volume of 16  $\mu$ l per well, add the same volume of TE to the blank control, and then add the prepared standards and diluted samples to the corresponding reaction wells in a volume of 4  $\mu$ l/well. It is recommended to use 20  $\mu$ l reaction system, if you need to carry out a smaller system reaction, the system components can be reduced in equal proportion.

### qPCR reaction program

Steps	Temperature	time	Cycles
Pre denaturation	95° C	10min	1
denaturation	95° C	10sec	40
Annealing/Extension	62° C	30sec	

If the average length of the library is greater than 700bp, the annealing/extension time should be increased appropriately.

Refer to the specific instrument setup program for dissolution curves.

## data analysis

### Standard curve production

The standard curve was plotted according to the data processing Excel sheet. The correlation coefficient R<sup>2</sup> of the standard curve should be not less than 0.99, and the slope should be located between -3.1 and -3.6 when the Ct value is the longitudinal coordinate. If the parameters of the standard curve are unreasonable, it is recommended to repeat the experiment.

DNA Standard name	DNA Standard concentration
DNA Standard A	60pM



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DNA Standard B	6pM
DNA Standard C	0.6pM
DNA Standard D	0.06pM
DNA Standard E	0.006pM

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### **Library Concentration Calculations**

The difference in Ct between the three replicate wells of the experiment should be no more than 0.2, otherwise the invalid data should be deleted or the experiment should be repeated. Do not use the Ct outside the valid Ct range of the standard curve to calculate the concentration of the diluted libraries. Please refer to the data processing Excel of this product for the specific library concentration calculation method.

### **matters needing attention**

These instructions should be read in detail before testing. It should be carried out by personnel with specialized experience or qualified by training. Mix gently by turning up and down, avoid foaming as much as possible, and centrifuge for a short time before use.

Avoid repeated freezing and thawing of this product; repeated freezing and thawing may degrade product performance.

When preparing reaction solutions, use new or non-contaminated tips and centrifuge tubes to prevent contamination as much as possible.