

Human Genomic DNA Quantification Kit

Project No. : H665940

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	H665940-1ml	H665940-5ml
2×GoldStar TaqMan Mixture	1ml	5 x 1ml
Primer Mix 1	300μl	5 x 300μl
Human DNA Standard 1 (100ng/μl)	100μl	5 x 100μl
50×High rox	40μl	200μl

Product Introduction

This product is a real-time fluorescence quantitative PCR (qPCR) using the probe method, which realizes the accurate detection of the concentration and quality of DNA extracted from various samples (paraffin samples, flow-sorted cells, serum or plasma samples and a small number of clinical samples, etc.). The product provides a full set of reagents required in the qPCR process, including reaction mix, primer mixtures, and standards. Simply add the extracted DNA and start the experiment, which is easy and convenient to operate and saves time and labor. The reaction mixture uses highly efficient and fast hot-start GoldStar Taq DNA Polymerase, which has high amplification sensitivity, good specificity and shortens the program reaction time.

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 LightCycler 96, Bio-rad iCycler 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, ViiA 7 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 3.

Applicable scope

This product is suitable for quantitative detection of human genomic DNA sample concentration in scientific research, clinical, forensic medicine and paternity testing.

Usage

1. Amplification template preparation

The 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 0.05-10 ng/μl. The samples were placed on ice at 4° C and set aside.

2. Standard dilution: according to the following table, firstly dilute Human DNA Standard 1 (100ng/ul) with TE to make 5 different concentrations of standard according to the table below. 10ng/ μ l of DNA Standard 1 (Std.1) can be stored stably at -20°C for 1 month; Std2-5 can only be used on the same day, and should be placed at 4°C or on ice when not in use for the time being after preparation. When not used temporarily after preparation, it should be kept at 4°C or on ice.

style	Corresponding concentration (ng/ μ l)	Minimum dilution volume (in μ l)
Std. 1	10	10[100ng/ μ l DNA Standard 1]+90TE
Std. 2	2.5	20[Std. 1]+60TE
Std. 3	0.625	20[Std. 2]+60TE
Std. 4	0.15625	20[Std. 3]+60TE
Std. 5	0.0390625	20[Std. 4]+60TE

3. qPCR reaction system preparation

The cryopreserved reagents to be used were completely melted and mixed by inversion several times before preparation, and then briefly centrifuged and prepared for use. 20 μ l of the base reaction system was as follows.

The base reaction system for 20 μ l was as follows:

reagents	20 μ l reaction system
2 \times GoldStar TaqMan Mixture	10 μ l
Primer Mix	3 μ l
Template	4 μ l
ddH ₂ O	3 μ l

Note: High Rox model: add 1 μ l of 50 \times High Rox per 50 μ l of reaction system; Low Rox model: add 1 μ l of 50 \times High Rox per 500 μ l of reaction system.

A sufficient amount of reaction system mixture was prepared according to the need, and after the reaction system was prepared and mixed thoroughly, it was added to the reaction wells in a volume of 16 μ l per well. Then add the prepared standards and diluted samples into the corresponding reaction wells, the amount added is 4 μ l/well. TE was added to the blank control tube, and the same amount was added at 4 μ l/well.

It is recommended to use 20 μ l of reaction, if you need to carry out a smaller system reaction, the system components can be reduced in equal proportion.

4. qPCR reaction program

The PCR mix in this kit contains a FAM fluorescent probe for the target gene and a VIC fluorescent probe with internal reference to Internal PCR Control (IPC). qPCR program with dual fluorescence of hydrolyzed probes needs to be selected for the assay. Please set up the program according to the instructions of your instrument.

PCR reaction temperature conditions were as follows:

move	temp	timing	circulate
premutability	95 $^{\circ}$ C	10min	1
denaturation	95 $^{\circ}$ C	10sec	
Annealing/Extension	60 $^{\circ}$ C	30sec	55

data analysis

1. Standard curve production

The standard curve was plotted with reference to the Excel sheet for data processing. The correlation coefficient R² of the standard curve should be not less than 0.98, and the slope should be located between -3.1 and -3.6 when the Ct value is used as 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 (ng/μl)
DNA Standard 1	10
DNA Standard 2	2.5
DNA Standard 3	0.625
DNA Standard 4	0.15625
DNA Standard 5	0.0390625

2. Analysis of results and calculation of concentrations

The Ct difference between experimental replicate wells for FAM signaling of the target gene should be no more than 0.3, otherwise invalid data need to be deleted or the experiment needs to be repeated, do not use Ct outside the valid Ct range of the standard curve to calculate the concentration of the sample. Specific calculations of sample concentrations were made with reference to the data processing Excel sheet.

If the FAM signal is abnormal, the VIC signal of the internal reference Internal PCR Control (IPC) should be analyzed to confirm whether the PCR reaction process is abnormal. If the Ct value of the VIC signal of the sample wells is significantly larger than that of the standard or blank control wells, it means that the sample has inhibited the PCR reaction.

matters needing attention

1. Before testing, these instructions should be read in detail. It should be operated by personnel with professional experience or qualified by training.
2. For use, please mix gently by turning up and down, avoid foaming as much as possible, and use it after centrifugation for a short period of time.
3. Avoid repeated freezing and thawing of the product, repeated freezing and thawing may degrade the performance of the product.
4. When preparing the reaction solution, please use new or non-contaminated tips and centrifuge tubes to try to prevent contamination.