Y Human Male DNA Quantification Kit

Item No.:Y665957

Storage conditions: -20 ℃, 12 months, such as the need for frequent use, can be stored in 2-8 ℃, try to avoid repeated freezing and thawing

Product content

Component	Y665957-1ml	Y665957-5ml
2×GoldStar Probe Mixture	1mL	5×1mL
Probe Primer Mix	300μL	5 x 300μL
Human DNA Standard (100ng/μl)	100μL	5 x 100μL
50 x High ROX	40μL	200μL

Product Introduction

This product is a real-time fluorescence quantitative PCR kit for detecting the concentration of human male Y chromosome, including carefully optimized PCR reaction solution, primer mixture and standards, especially suitable for the quantitative detection of precious and micro DNA samples. The kit adopts a new efficient and fast hot-start amplification enzyme GoldStar Taq DNA Polymerase, which effectively avoids non-specific amplification caused by non-specific binding of primers and templates or primer dimerization at room temperature. This product realizes accurate quantification of Y chromosome and can be applied in various fields such as genetic mapping, species polymorphism research, disease gene localization, paternity testing and forensic analysis. 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, 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.

Scope of application

This product is suitable for quantitative testing of male Y chromosome DNA in scientific research, clinical, forensic medicine and paternity testing.

Usage

1. 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 0.05-10 ng/μL. 4° C on ice was set aside.
2. Standard dilution: according to the following table, firstly dilute Human DNA Standard (100ng/uL) with TE to make 5 standards of different concentrations

according to the table below. 10ng/μL of DNA Standard 1 (Std.1) can be stored stably at -20℃ for 1 month; Std2-5 can only be used on the same day, and should be placed at 4℃ or on ice when not in use for the time being after preparation. When Std2-5 are not used temporarily after preparation, they should be stored at 4℃ or on ice.

style	Corresponding concentration (ng/μl)	Minimum dilution volume (in μl)
Std.1	10	10[100ng/μl DNA Standard]+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

Before preparation, the cryopreserved reagents to be used were completely melted and mixed by inverting several times, then centrifuged briefly and prepared. Standards and templates were diluted as described above and prepared. The base reaction system for 20 μL was as follows:

reagents	20μl reaction system
2×GoldStar Probe Mixture	10μl
Probe Primer Mix	3μl
Template	4 μl
ddH_2O	3μl

Note: High ROX model: add 1 μL of 50×High ROX per 50 μL of reaction system; Low ROX model: add 1 μL of 50×High ROX per 500 μL 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 μl per well. Then add the prepared standards and diluted samples into the corresponding reaction wells, the amount of addition is 4μL/well. TE was added to the blank control tube, and the same amount was added at 4 μL/well.

It is recommended to use 20 μL for the reaction, if you need to perform a smaller system reaction, reduce the system components in equal proportion.

4. qPCR reaction program

The PCR mix of 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 follow the instructions of the instrument used to set up the qPCR program, and the PCR temperature conditions are as follows:

Steps	Temperature	time	Cycles
Pre denaturation	95° C	10min	1
denaturation	95° C	10sec	40
Annealing/Extension	60° C	30sec	

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. For specific calculations, please refer to the data processing Excel for this product.

If the FAM signal is abnormal, the VIC signal of the internal reference Internal PCR Control (IPC) needs to be analyzed to confirm whether the PCR reaction process is abnormal. If the Ct value of the sample null VIC is significantly larger than that of the standard or blank control wells, it means that the sample inhibits 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 prevent contamination as much as possible.