



Y-chromosome Human DNA Quantitative Kit

Product Number: PCK852

Shipping and Storage

-20 °C, 12 months. If frequently used, it can be stored at 2-8 °C to avoid repeated freeze-thaw as much as possible.

Components

Component	PCK852	PCK852
2×GoldStar Probe Mixture	1 mL	5×1 mL
Probe Primer Mix	300 µL	5×300 µL
Human DNA Standard (100 ng/µl)	100 µL	5×100 µL
50×High ROX	40 µL	200 µL

Description

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 standard samples, especially suitable for quantitative detection of precious and trace DNA samples. The reagent kit adopts a new, efficient and fast hot start amplification enzyme Golden Star Taq DNA Polymerase, effectively avoiding non-specific amplification caused by non-specific binding of primers and templates or primer dimers under room temperature conditions. This product achieves accurate quantification of the Y chromosome and can be applied in various fields such as genetic mapping, species polymorphism research, disease gene mapping, parent-child identification, and forensic analysis.

ROX dye is used to correct the fluorescence signal error generated between wells in quantitative PCR instruments, and is generally used in Real Time PCR amplification instruments from companies such as ABI and Stratagene. The excitation optical systems of different instruments vary, so the concentration of ROX dye must be matched with 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 that require 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 etc.

Instruments that require High ROX calibration: ABI Prism 7000/7300/7700/7900, Eppendorf, ABI StepOne/Step One Plus etc.

Note: The preparation methods for High Rox and Low Rox are described in protocol Step 3.

Application

This product is suitable for quantitative detection of male Y chromosome DNA in fields such as scientific research, clinical practice, forensic science, and parent-child identification.

Protocol

1. Preparation of amplification template

Dilute the sample to be tested with TE (10 mM Tris Cl, pH8.0, 1mM EDTA), and try to maintain a concentration between 0.05 and 10 ng/µl. Place on ice at 4°C for backup.

2. Standard dilution: According to the following table, first dilute Human DNA Standard 1 (100ng/µl) with TE to obtain 5 different concentrations of standard samples. 10 ng/µl of DNA Standard 1 (Std. 1) can be stably stored at -20 °C for 1 month; Std2-5 can only be used on the same day, and should be stored at 4°C or on ice when not in use temporarily after preparation.

Standard sample	Corresponding concentration(ng/µl)	Minimum Dilution Volume(µl)
Std.1	10	10 [100 ng/µl DNA Standard 1]+ 90 TE
Std.2	2.5	20 [Std. 1] +60 TE

Std.3	0.625	20 [Std. 2] +60 TE
Std.4	0.15625	20 [Std. 3] +60 TE
Std.5	0.0390625	20 [Std. 4] +60 TE

3. Preparation of qPCR reaction system

Before preparation, completely melt the required cryopreserved reagents and mix them multiple times, then centrifuge briefly for later use. The basic reaction system for 20 μ l is as follows.

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Reagent	20 μ l Reaction system
2 \times GoldStar Probe Mixture	10 μ l
Probe Primer Mix	3 μ l
Template	4 μ l
ddH ₂ O	3 μ l

Note: High Rox models: add 1 μ l of 50 \times High Rox to every 50ml reaction system ; Low Rox model: add 1 μ l 50 \times High Rox to every 500 μ l reaction system.

Prepare a sufficient amount of reaction system mixture as needed. After the reaction system is prepared and thoroughly mixed, add 16 μ l of volume per well to the reaction pores. Then add the prepared standard and diluted samples to the corresponding reaction wells at a rate of 4 μ l/well. Add TE to the blank control tube at a rate of 4 μ l/well. It is recommended to use 20 μ l of the reaction. If a smaller system reaction is required, the components of the system can be reduced proportionally.

4. qPCR reaction program

The PCR mix of this kit contains FAM fluorescent probes for the target gene and VIC fluorescent probes with internal reference to the PCR Control (IPC). When detecting, it is necessary to select the qPCR program with dual fluorescence of the hydrolysis probe. Please set according to the instructions of the instrument used.

The temperature conditions for PCR reaction are as follows:

Step	Temperature	Time	Cycles
Pre denaturation	95 $^{\circ}$ C	10 min	1
Denaturation	95 $^{\circ}$ C	10 sec	40
Annealing/Extension	60 $^{\circ}$ C	30 sec	

Data Analysis

1. Standard curve production

Draw standard curves based on data processing Excel tables. The correlation coefficient R² of the standard curve should not be less than 0.98. When using the Ct value as the ordinate, the slope should be between -3.1 and -3.6. If the parameters of the standard curve are unreasonable, it is recommended to repeat the experiment.

DNA Standard	DNA Standard concentration (ng/ μ l)
DNA Standard 1	10
DNA Standard 2	2.5
DNA Standard 3	0.625
DNA Standard 4	0.15625
DNA Standard 5	0.0390625

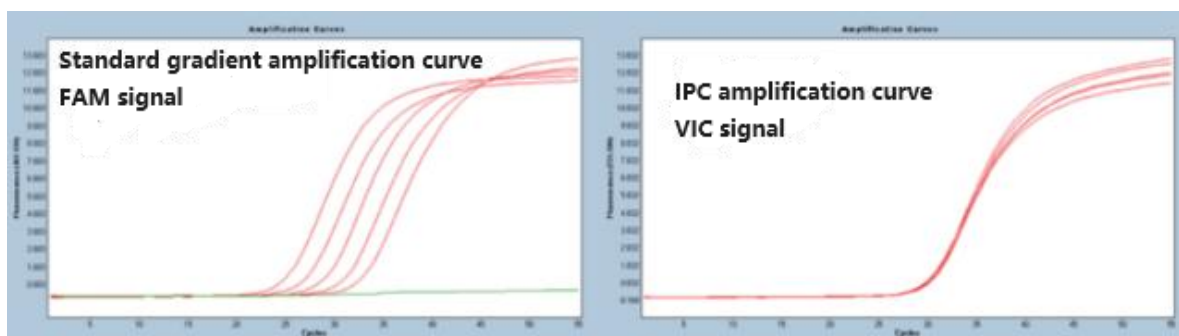
2. Result analysis and concentration calculation

The Ct difference between the experimental wells of the target gene FAM signal should not exceed 0.3, otherwise invalid data or repeated experiments need to be deleted. Do not use Ct outside the effective Ct range of the standard curve to calculate the concentration of the sample.

The specific calculation of sample concentration refers to the data processing Excel table.

If the FAM signal is abnormal, it is necessary to analyze the VIC signal of the Internal PCR Control (IPC) to confirm whether the PCR reaction process is abnormal. If the Ct value of the VIC signal in the sample well is significantly higher than that of the

standard or blank control well, it indicates that the sample has inhibition of PCR reaction.



Note

1. Before the experiment, this instruction should be read in detail. The operation should be carried out by personnel with professional experience or qualified training.
2. Please gently mix it upside down to avoid foaming, and use after briefly centrifuging.
3. Avoid repeated freeze-thaw of this product, as repeated freeze-thaw may cause a decrease in product performance.
4. When preparing the reaction solution, please use a new or non-polluting gun head and centrifuge tube to prevent contamination as much as possible.