**Tinzyme Co., Limited**

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

# Human Genomic DNA Quantitative Kit

**Product Number: PCK83**

## Shipping and Storage

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

## Components

| Component | PCK83 | PCK83 |
|---|---|---|
| 2×GoldStar TaqMan Mixture | 1ml | 5×1 ml |
| Primer Mix 1 | 300µl | 5×300 µl |
| Human DNA Standard 1(100ng/µl) | 100µl | 5×100 µl |
| 50×High rox | 40µl | 200 µl |

## Description

This product uses the probe method for real-time fluorescence quantitative PCR (qPCR) to accurately detect the concentration and quality of DNA extracted from various samples (paraffin samples, flow cytometry sorted cells, serum or plasma samples, and small amounts of clinical samples, etc.). The product provides a complete set of reagents required for the qPCR process, including reaction mixture, primer mixture, and standard samples. Simply add the extracted DNA to start the experiment, which is simple, convenient, time-saving, and labor-saving. The reaction mixture uses an efficient and fast hot start Golden Star Taq DNA Polymerase, which has high amplification sensitivity and specificity, and shortens the time of the programmed reaction.

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 LightCyler 96，Bio-rad iCyler 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 Operation Step 3.

## Application

This product is suitable for quantitative detection of human genomic DNA sample concentration 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℃ 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 ℃ for 1 month; Std2-5 can only be used on the same day, and should be stored at 4℃ 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 20μl is as follows.

The basic reaction system for 20μl is as follows:

| Reagent | 20μl Reaction system |
|---|---|
| 2×GoldStar TaqMan Mixture | 10μl |
| Primer Mix | 3μl |
| Template | 4μl |
| $ddH_2O$ | 3μl |

Note:High Rox models: add 1μl of 50×High Rox to every 50ml reaction system ; Low Rox model: add 1μl 50×High Rox to every 500μl reaction system.

Prepare a sufficient amount of reaction system mixture as needed. After the reaction system is prepared and thoroughly mixed, add 16μl 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 4μl/well. Add TE to the blank control tube at a rate of 4μl/well. It is recommended to use 20μl 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℃ | 10 min | 1 |
| Denaturation | 95℃ | 10 sec | 55 |
| Annealing/Extension | 60℃ | 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/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 |

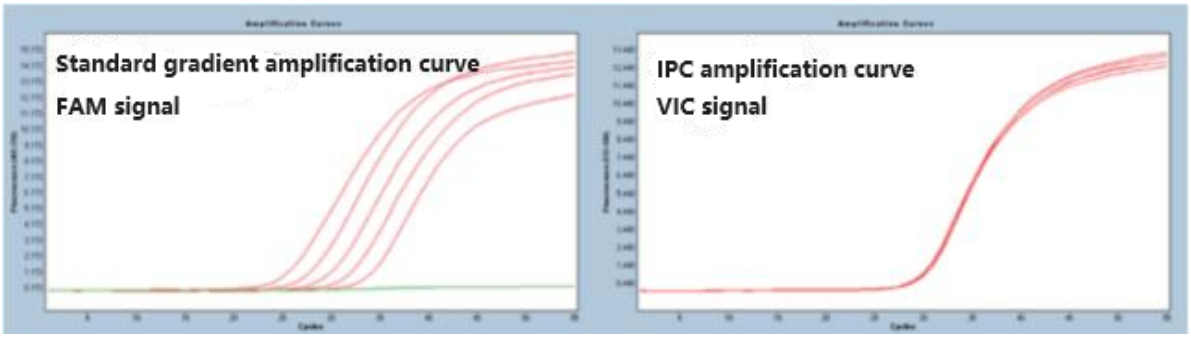
2. Result analysis and concentration calculation

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. Plea se 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.