

Instructions for Use of Human Residual DNA Detection Kit (qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- HD001

Introduction

Human residual DNA detection kit is a kit specially designed for quantitative detection of human (host) DNA in intermediate products, bulk products and final products of various biological products.

This kit quantitatively detects human residual DNA in samples based on Taqman probe principle. The kit is a rapid, specific and reliable device, with the minimum detection limit reaching fg level.

Specification

100 Reactions.

Main components

Components	Specification	Storage conditions
Human DNA quantitative reference	50 μ L \times 1 vial	-18 $^{\circ}$ C and below
Human Primer&Probe MIX	550 μ L \times 1 vial	-18 $^{\circ}$ C and below, protected from light
2x qPCR Reaction Buffer	1.1 mL \times 1 vial	-18 $^{\circ}$ C and below, protected from light
DNA diluent	1.0 mL \times 3 vials	-18 $^{\circ}$ C and below
ROX High*	50 μ L \times 1 vial	-18 $^{\circ}$ C and below, protected from light
ROX Low*	50 μ L \times 1 vial	-18 $^{\circ}$ C and below, protected from light

* Please select appropriate ROX for corresponding model.

Table 1: Kit components

Storage conditions and shelf life

The shelf life is 18 months when stored at -18 $^{\circ}$ C and below.

Applicable models (including but not limited to)

- ◆ ABI PRISM 7500
- ◆ CFX96(Bio-Rad)
- ◆ Linegene 9600plus (Bioer)

When using with different instrument models, please select the appropriate reference stain ROX.

Instruments	ROX reference stain
Applied Biosystems® 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™	ROX High
Applied Biosystems® 7500, ViiA™ 7, QuantStudio™ 12K Flex, Agilent Mx3000P™, Mx3005P™, and Mx4000™	ROX Low
Rotor-Gene™, DNA Engine Opticon™, Opticon™ 2, Chromo 4™ Real-Time Detector, Mastercycler® ep realplex, Smart Cycler®, Roche LightCycle®480, Roche LightCycler®Nano, Bio-Rad CFX96, and Illumina Eco™	No ROX

Prepared by the user

- ◆ 1.5 mL low-attachment sterile centrifuge tube
- ◆ qPCR plate or strip tubes

Operation procedures

I. Dilution of Human DNA Quantitative Reference and Preparation of Standard Curve

Label the concentration of Human DNA quantitative reference on the tube wall label at 30 ng/μL. Please confirm before dilution.

1. Remove Human DNA quantitative reference and DNA diluent from the package and thaw on ice; after thawing completely, shake gently to mix well, and centrifuge instantly.
2. Take 6 clean 1.5 mL centrifuge tubes and label them as STD0, STD1, STD2, STD3, STD4, and STD5, respectively.
3. Dilute the Human DNA quantitative reference by 10 folds with DNA diluent in the STD0 tube to obtain STD0. Shake to mix well and quickly centrifuge briefly for 10 s.
4. Add 90 μL of DNA diluent to STD1, STD2, STD3, STD4, and STD5 tubes, respectively.
5. The standard dilution process is shown in the table below:

Standard No.	Dilution volume	Concentration (fg/μL)
STD1	10 μL STD0 + 90 μL DNA diluent	3.00×10 ⁵
STD2	10 μL STD1 + 90 μL DNA diluent	3.00×10 ⁴
STD3	10 μL STD2 + 90 μL DNA diluent	3.00×10 ³
STD4	10 μL STD3 + 90 μL DNA diluent	3.00×10 ²
STD5	10 μL STD4 + 90 μL DNA diluent	3.00×10 ¹

Table 2. Dilution of standards

II. Preparation and addition of qPCR reaction solution

1. Calculate the required number of reaction wells based on the standard curve and the number of samples to be tested. Generally, 3 replicate wells are required for each sample.

Number of reaction wells = (5 concentration gradients on the standard curve + 1 no template control (NTC) + number of test samples) × 3.

2. Calculate the total amount of qPCR MIX required for this time based on the number of reaction wells: qPCR MIX = (number of reaction wells + 2) × 15 μL (including loss in 2 wells)
3. Take out each reagent and place on ice to thaw, gently shake to mix well, and prepare qPCR MIX according to the table below:

Components	Reaction in single-well
2x qPCR Reaction Buffer	10 μL
Human Primer&Probe MIX	4.6 μL
ROX*	0.4 μL
Total volume	15 μL

* Please select appropriate ROX for corresponding model. If there is no ROX suitable for the model, please add deionized water (free of nucleic acid and nuclease contamination) of same volume.

The loading to each reaction well is as follows:

Standard curve	15 μL qPCR MIX +5 μLSTD1/STD2/STD3/STD4/STD5
No template control (NTC)	15 μL qPCR MIX + 5 μL DNA diluent
Test sample	15 μL of qPCR MIX + 5 μL of DNA extract of the test sample

The total liquid volume in each well should be 20 μL after loading. Gently shake the 96-well plate to mix well, perform rapid centrifugation for 10 seconds, and place the plate in the qPCR system.

III. qPCR program parameter setting

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Create an experimental reaction program and set up a two-step reaction program. The PCR reaction program is as follows: Pre-denaturation at 95°C for 10 minutes, at 95°C for 10 seconds, and at 60°C for 15 seconds; repeat for 40 cycles, with the reaction volume of 20 μL.
2. Create an experimental reaction plate, click on "Select Fluorophores" and select fluorescent FAM.

In the reaction plate diagram, select the Sample well, pull down in Sample Type to select Unknow, check the fluorescence FAM, Target Name is designated as Human-DNA, and input the number of replicates per sample and Sample Name.

In the reaction plate chart, select the Standard well, pull down in Sample Type to select Standard, check the fluorescence FAM, Target Name is designated as Human-DNA, and input the number of replicates for each dilution

gradient and Sample Name. And Concentration column of STD1, STD2, STD3, STD4 and STD5 is assigned with values of 300000, 30000, 3000, 300 and 30, respectively, and select the unit of fg.

3. Click Start Run to select the save path

IV. qPCR result analysis

1. Click "Quantitation" on the data analysis window to read the slope, intercept, amplification effect, and R^2 of the standard curve.
2. In the window Quantitation Data, the SQ Mean column reads the test values of the no-template control (NTC) and the test sample in fg/ μ L.
3. NTC test results shall be N/A or Ct value is greater than Ct mean of the lowest concentration of the standard curve.

Disclaimer

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