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

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

Cat. No. HG- HF001

Introduction

This kit is a kit specially designed for quantitative detection of the size distribution of residual human (host) DNA fragments in intermediates, bulk products and final products of various biological products.

This kit is based on the principle of PCR fluorescence probe method to quantitatively detect the size distribution of residual human (host) DNA fragments in samples. This kit designs three different amplified fragments (99 bp, 200 bp and 307 bp). The human DNA quantitative reference is used to generate standard curves for different amplified fragments, and the fragment distribution of residual human DNA in samples is analyzed by the ratio of different size fragments.

Specification

3×100 Reactions

Main components

Table 1: Kit components

Serial Number	Components	Fill volume/vial	Quantity	Storage conditions
1	Human Primer&Probe MIX-99	300 μL	1	-18℃, protected from light
2	Human Primer&Probe MIX-200	300 μL	1	-18℃, protected from light
3	Human Primer&Probe MIX-307	300 μL	1	-18℃, protected from light
4	Human DNA quantitative reference	60 μL	1	-18℃
5	2×Probe qPCR Mix	1.25 mL	4	-18℃
6	DNA diluent	1 mL	4	-18℃
7	IPC Mix	450 μL	1	-18℃, protected from light
8	ROX High*	50 μL	1	-18℃, protected from light
9	ROX Low*	50 μL	1	-18℃, protected from light

* Please select appropriate ROX for corresponding model.

Storage conditions and shelf life

The shelf life is 18 months when stored at -18℃ and below.

Applicable models (including but not limited to)

- ABI PRISM 7500
- CFX96(Bio-Rad)
- FQD-96A (Bioer)
- Roche Light Cycler 480

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 or 2 mL sterile low-attachment centrifuge tubes
- 96-well qPCR plate or 8-strip PCR tubes compatible with the PCR system
- Sterile low-attachment pipette tips with cartridge, 1000 μL, 200 μL, and 10 μL specifications
- Centrifuge
- Shaker
- Pipettes of various specifications (such as 1000 μL, 200 μL, 10 μL, and 2.5 μL).
- Fluorescent quantitative PCR system

Operation procedures

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

Human DNA quantitative reference (concentration: 3 ng/μL) is subjected to gradient dilution with DNA diluent provided in the kit at concentrations of 300 pg/μL, 30 pg/μL, 3 pg/μL, 300 fg/μL and 30 fg/μL. The detailed procedures are as follows:

1. Place the Human DNA quantitative reference and DNA diluent in the kit on ice; after thawing completely, shake gently to mix well, and centrifuge briefly to settle the liquid to the bottom of the tube.
2. Take five 1.5-mL clean centrifuge tubes and label as ST1, ST2, ST3, ST4 and ST5, respectively.
3. Add 180 μL of DNA diluent into ST1, ST2, ST3, ST4 and ST5 tubes, respectively.
4. Perform 5 dilutions according to Table 2, ensure thorough mixing after each dilution, and then perform the next gradient dilution. For example, add 20 μL of Human DNA quantitative reference to the prepared ST1 tube in Step 3, shake gently to mix well, centrifuge briefly to settle the liquid to the bottom of the tube; then add 20 μL of standard in ST1 to the prepared ST2 tube in Step 3……dilute sequentially.

Table 2. Dilution of standards

Standard No.	Dilution volume	Concentration
ST1	180 μL of DNA diluent + 20 μL of Human DNA quantitative reference	300 pg/μL
ST2	180 μL of DNA diluent + 20 μL of ST1	30 pg/μL
ST3	180 μL of DNA diluent + 20 μL of ST2	3 pg/μL
ST4	180 μL of DNA diluent + 20 μL of ST3	300 fg/μL
ST5	180 μL of DNA diluent + 20 μL of ST4	30 fg/μL

II. Preparation of Negative Control Sample (NCS)

Add 100 μL of sample matrix solution (or DNA diluent) to 1.5-mL clean centrifuge tube, and label it as negative control sample (NCS).

The NCS can be pre-treated together with the test samples from the same batch to prepare the NCS purified solution.

Note: To meet the need for simultaneous analysis of fragments with three different amplification lengths, ≥ 100 μL of DNA elution volume is required for sample pre-treatment.

III. Preparation of qPCR Reactions

1. Take out each reagent from the freezer and place them on ice.
2. 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. That is, the number of reaction wells = (standard curve of 5 concentration gradients + 1 NTC + 1 NCS + number of test samples) × 3.

3. Calculate the total amount of qPCR reaction solution required for this time based on the number of reaction wells: QPCR reaction solution = (Number of reaction wells + 2) × 20 μL (containing loss in 2 wells).
4. After each reagent is fully thawed on ice, the qPCR reaction mixture corresponding to the amplified fragment is prepared by combining the number of reaction wells as shown in Tables 3, 4, and 5 and mixed well.

Table 3 Preparation of qPCR Reaction Mixture for 99-bp Fragment

Components	Reaction in single-well
2×Probe qPCR Mix	15 μL
Human Primer&Probe MIX-99	3 μL
IPC Mix	1.4 μL
ROX*	0.6 μL
Total volume	20 μL

Table 4 Preparation of qPCR Reaction Mixture for 200-bp Fragment

Components	Reaction in single-well
2×Probe qPCR Mix	15 μL
Human Primer&Probe MIX-200	3 μL
IPC Mix	1.4 μL
ROX*	0.6 μL
Total volume	20 μL

Table 5 Preparation of qPCR Reaction Mixture for 307-bp Fragment

Components	Reaction in single-well
2×Probe qPCR Mix	15 μL
Human Primer&Probe MIX-307	3 μL
IPC Mix	1.4 μL
ROX*	0.6 μL
Total volume	20 μL

* Select suitable ROX for corresponding model; if the model is suitable for no ROX, add an equal volume of deionized water (it is required to add deionized water without nucleic acid and nuclease contamination).

IV. Loading

1. Mix each qPCR reaction mixture prepared by gentle shaking and centrifuge briefly to settle the liquid to the bottom of the tube. Add into the corresponding wells at 20 μL/well in typesetting design (as shown in Table 9 or adjusted by personalized design), select the corresponding amplified fragments and load as shown in Tables 6, 7 and 8, with a total volume of 30 μL per well after loading.

Table 6 Example of Loading of 99-bp Fragment in Each Reaction Well

ST-99bp	20 μL of qPCR reaction mixture for 99-bp fragment + 10 μL of ST1/ST2/ST3/ST4/ST5
NTC	20 μL of qPCR reaction mixture for 99-bp fragment + 10 μL of DNA diluent
NCS	20 μL of qPCR reaction mixture for 99-bp fragment + 10 μL of NCS purified solution
Test sample	20 μL of qPCR reaction mixture for 99-bp fragment + 10 μL of test sample

Table 7 Example of Loading of 200-bp Fragment in Each Reaction Well

ST-200bp	20 μL of qPCR reaction mixture for 200-bp fragment + 10 μL of ST1/ST2/ST3/ST4/ST5
NTC	20 μL of qPCR reaction mixture for 200-bp fragment + 10 μL of DNA diluent
NCS	20 μL of qPCR reaction mixture for 200-bp fragment + 10 μL of NCS purified solution
Test sample	20 μL of qPCR reaction mixture for 200-bp fragment + 10 μL of test sample

Table 8 Example of Loading of 307-bp Fragment in Each Reaction Well

ST-307bp	20 μL of qPCR reaction mixture for 307-bp fragment + 10 μL of ST1/ST2/ST3/ST4/ST5
NTC	20 μL of qPCR reaction mixture for 307-bp fragment + 10 μL of DNA diluent
NCS	20 μL of qPCR reaction mixture for 307-bp fragment + 10 μL of NCS purified solution
Test sample	20 μL of qPCR reaction mixture for 307-bp fragment + 10 μL of test sample

Table 9. Example of a 96-well plate layout

99bp				200bp				307bp				
NTC	NTC	NTC		NTC	NTC	NTC		NTC	NTC	NTC		A
NTC	NTC	NTC		NTC	NTC	NTC		NTC	NTC	NTC		B
S1	S1	S1	S2	S1	S1	S1	S2	S1	S1	S1	S2	C
ST1	ST1	ST1	S2	ST1	ST1	ST1	S2	ST1	ST1	ST1	S2	D
ST2	ST2	ST2	S2	ST2	ST2	ST2	S2	ST2	ST2	ST2	S2	E
ST3	ST3	ST3	S3	ST3	ST3	ST3	S3	ST3	ST3	ST3	S3	F
ST4	ST4	ST4	S3	ST4	ST4	ST4	S3	ST4	ST4	ST4	S3	G
ST5	ST5	ST5	S3	ST5	ST5	ST5	S3	ST5	ST5	ST5	S3	H
1	2	3	4	5	6	7	8	9	10	11	12	

& This example represents a DNA standard curve of samples at five concentration gradients, a NTC, a NCS, and three test samples. prepare wells in triplicate. It can also be designed and typeset according to the user's own commonly used practice.

2. Seal the 96-well plate with an optical plate sealer, gently shake to mix well, perform rapid centrifugation with a centrifuge dedicated for 96-well plate to let all liquid gather at tube bottom, then place the plate in the qPCR system.

V. Run

ABI 7500 qPCR instrument (software V2.0.6) is used as an example.

1. Create a blank new procedure and select Absolute Quantitative Test Template and Taqman Probe Method.
2. Three sets of qPCR reactions are used to create new detection probes named as 99 bp, 200 bp, 307 bp, select reporter fluorophore as FAM and quench fluorophore as none; new detection probes are created and named as IPC, select reporter fluorophore as VIC, quench fluorophore as none, and detection reference fluorescence is ROX.
3. Select standard wells in the plate as Standard, assign values of 300, 30, 3, 0.3, 0.03 (in pg/μL), and name as ST1, ST2, ST3, ST4, ST5 in the corresponding Sample Name column; select NTC as Negative Control, NCS and test sample wells as Unknown, and name as NTC, NCS, S1, S2, S3 in the corresponding Sample Name column.
4. Set up three-step reaction program: 95 ℃ for 5 min; 45 cycles of 95 ℃ for 15 s, 58 ℃ for 30 s, 72 ℃ for 1 min; and a reaction volume of 30 μL.
5. Run the PCR program.

VI. qPCR result analysis

1. In the "Amplification Plot" of Results, the user may initially check whether the amplification curve is of normal shape. Usually, the system will automatically set the threshold and baseline. Multiple thresholds may be generated in case of different target settings, leading to inaccurate result analysis. In such case, please manually set the threshold line, which must be within the exponential amplification region, for example, the threshold is usually set to 0.15. Select "Auto Baseline", and click "Analyze" to view the adjusted results.
2. In Standard Curve in Results, the slope, intercept, R^2 of the standard curve can be read.
3. In the Report in Results, the Mean Quantity column reads the test values in pg/μL for the NTC, NCS, and test samples (associated with the assignment of standards in 5.3). The Ct value of NTC and NCS should be greater than the Ct value of ST5, and the detection value should be less than 30 fg/μL.
4. Calculate the percentage of test samples for the 200-bp fragment and the 307-bp fragment using the test value of the 99-bp fragment as 100%.
5. Normally, Ct values of the IPC should be comparable for each well. When the Ct value of the IPC in the sample well is more than 1 greater than the Ct value in the standard curve or NTC, it suggests that there may be residues present in the sample that inhibit the PCR reaction.

Disclaimer

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