

Instructions for Use of HEK293 Cell Residual DNA Fragment Analysis Detection Kit (qPCR)

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

Cat. No. HG- HF002

Introduction

This kit is a kit specially designed for quantitative detection of the size distribution of residual HEK293 Cell 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 HEK293 Cell DNA fragments in samples. This kit designs three different amplified fragments (99 bp, 200 bp and 307 bp). The HEK293 Cell DNA quantitative reference is used to generate standard curves for different amplified fragments, and the fragment distribution of residual HEK293 Cell 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	HEK293 Primer&Probe MIX-99	300 µL	1	-18℃, protected from light
2	HEK293 Primer&Probe MIX-200	300 µL	1	-18℃, protected from light
3	HEK293 Primer&Probe MIX-307	300 µL	1	-18℃, protected from light
4	HEK293 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*	200 µL	1	-18℃, protected from light
9	ROX Low*	200 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
- ◆ FQD-96A (Bioer)
- ◆ CFX96(Bio-Rad)
- ◆ 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 LightCycler®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
- ◆ Fluorescent quantitative PCR system
- ◆ Centrifuge
- ◆ Shaker
- ◆ Pipettes of various specifications (such as 1000 µL, 200 µL, 10 µL, and 2.5 µL).

Operation procedures

I. Dilution of HEK293 DNA Quantitative Reference and Preparation of Standard Curve

HEK293 DNA quantitative reference (concentration: 3 ng/µL) is subjected to gradient dilution with DNA diluent provided in the kit at concentrations of 3×10^5 fg/µL, 3×10^4 fg/µL, 3×10^3 fg/µL, 3×10^2 fg/µL, 3×10^1 fg/µL. The detailed procedures are as follows:

- Place the HEK293 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.
- Take five 1.5mL clean centrifuge tubes and label as ST1, ST2, ST3, ST4 and ST5, respectively.
- Add 180 µL of DNA diluent into ST1, ST2, ST3, ST4 and ST5 tubes, respectively.
- 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 HEK293 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 HEK293 DNA quantitative reference	3×10^5 fg/µL
ST2	180 µL of DNA diluent + 20 µL of ST1	3×10^4 fg/µL
ST3	180 µL of DNA diluent + 20 µL of ST2	3×10^3 fg/µL
ST4	180 µL of DNA diluent + 20 µL of ST3	3×10^2 fg/µL
ST5	180 µL of DNA diluent + 20 µL of ST4	3×10^1 fg/µL

II. Preparation of Negative Control Sample (NCS and NTC)

Add 100 µL of sample matrix solution (or DNA diluent) to 1.5mL 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. When adding sample for qPCR reaction, add 10uL DNA diluent which is negative control NTC.

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 additive system

Standard recovery ERC: It is recommended to use 90ul sample + 10ulST3. It can also be prepared in other ways according to the actual situation.

Calculation of standard recovery rate: ERC recovery rate is between 50% and 150% (standard recovery rate = $\text{ERC}/(0.9 \times \text{sample} + 0.1 \times \text{ST3})$).

IV. Preparation of qPCR Reactions

- Take out each reagent from the freezer and place them on ice.
- 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.
- 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).
 - 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
HEK293 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
HEK293 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
HEK293 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).

V. Loading

- 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

& 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.

- 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.

Table 9. Example of a 96-well plate layout

99bp				200bp				307bp				
NTC	NTC	NTC		NTC	NTC	NTC		NTC	NTC	NTC		A
NCS	NCS	NCS		NCS	NCS	NCS		NCS	NCS	NCS		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	

VI. 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 3×10^5 , 3×10^4 , 3×10^3 , 3×10^2 , 3×10^1 (in fg/ μ L), 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 Unknow, and name as NTC, NCS, S1, S2, S3 in the corresponding Sample Name column.
4. Set up three-step reaction program: 95 $^{\circ}$ C for 5 min; 45 cycles of 95 $^{\circ}$ C for 15 s, 58 $^{\circ}$ C for 30 s, 72 $^{\circ}$ C for 1 min; and a reaction volume of 30 μ L.
5. Run the PCR program.

VII. 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. Data reliability evaluation:
 - The difference in Ct values between 3 replicate wells shall be less than 1.0, except for wells with Ct value greater than 35;
 - The CT values of negative controls NTC and NCS should be greater than the CT value of the lowest concentration of the standard curve, or the criteria should be set based on the laboratory's own validation results;
 - Linear correlation coefficient R^2 of the standard curve shall be equal or greater than 0.98, amplification efficiency shall be within 85%-110%;
 - The recovery of ERC shall be within 50%-150%.
3. The sample value of 99bp fragment is 100%, and the sample percentage of 200bp fragment and 307bp fragment is calculated.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

