

HEK293 Host Cell Residue DNA Size Analysis Kit

Product description

The HEK293 Host Cell Residue DNA Size Analysis Kit is designed for the quantitative analysis of HEK293 residual DNA fragments of various lengths in intermediates, semi-finished products, and final products of biological preparations.

This kit utilizes the qPCR fluorescence probe principle to specifically and rapidly detect HEK293 DNA residues both below and above 200 base pairs, with a quantification limit as low as 10 fg/ μ L. It also includes the HEK293 DNA Control (DNA quantitative reference). This kit can be used in conjunction with the company's magnetic bead-based residual DNA sample preparation kits (Cat#18461ES).

Specifications

Cat.No.	41316ES70-EN / 41316ES74-EN
Size	4 $\times$ 50 T-EN / 4 $\times$ 100 T-EN

Components

Components No.	Name	41316ES70-EN	41316ES74-EN
41316-A	HEK293 qPCR Mix	0.75 mL $\times$ 4	1.5 mL $\times$ 4
41316-B1	HEK293 Primer&Probe Mix-82	250 μ L	500 μ L
41316-B2	HEK293 Primer&Probe Mix-133	250 μ L	500 μ L
41316-B3	HEK293 Primer&Probe Mix-227	250 μ L	500 μ L
41316-B4	HEK293 Primer&Probe Mix-515	250 μ L	500 μ L
41316-C	DNA Dilution Buffer	1.8 mL $\times$ 2	1.8 mL $\times$ 4
41316-D	HEK293 DNA Control (30 ng/ μ L)	25 μ L	50 μ L

Storage

This product should be stored at -25~-15°C for 2 years.

Both 41316-A and 41316-B1&B2&B3&B4 all should be stored protected from light.

Applicable instrument models

Include but not limited to:

Bio-Rad: CFX96 Optic Module.

Thermo Scientific: ABI 7500; ABI Quant Studio 5.

Instructions

1. HEK293 DNA Standard dilution and Standard curve preparation

The HEK293 Fragment Analysis Kit includes four amplification fragments of different lengths: 82bp, 133bp, 227bp, and 515bp. When establishing standard curves, set up separate curve for each amplification fragment and calculate

their residual amounts and relative distributions based on the corresponding standard curves.

Use the DNA Dilution Buffer provided in the kit to perform a gradient dilution of the HEK293 DNA Control. The dilution concentrations should be: 3 ng/μL, 300 pg/μL, 30 pg/μL, 3 pg/μL, 300 fg/μL, and 30 fg/μL.

See detailed instructions below:

- 1) Thaw the HEK293 DNA control and DNA dilution buffer on ice. After completely thawed, vortex gently to mix, and centrifuge at low speed for 10 secs.
- 2) Take out seven clean 1.5 mL tubes, marked with Std0, Std1, Std2, Std3, Std4, Std5.
- 3) Add 90 μL DNA dilution buffer and 10 μL HEK293 DNA Control to the 1.5 mL microfuge tube labeled Std0, namely dilute to 3 ng/μL. Mix and then centrifuge for 10 secs. Subpackage the diluted DNA standard and it can be stored in the short term (no more than 3 months) at -25~-15°C. Please avoid repeated freeze-thaw.
- 4) Add 90 μL DNA dilution buffer into other tubes^{***}, then follow the below procedure for the serial dilutions^{****}.

Tube	Dilution Ratio	Standard concentration
Std1	10 μL Std0 + 90 μL DNA Dilution Buffer	300 pg/μL
Std2	10 μL Std1 + 90 μL DNA Dilution Buffer	30 pg/μL
Std3	10 μL Std2 + 90 μL DNA Dilution Buffer	3 pg/μL
Std4	10 μL Std3 + 90 μL DNA Dilution Buffer	300 fg/μL
Std5	10 μL Std4 + 90 μL DNA Dilution Buffer	30 fg/μL

Table1 Standard gradient dilution

^{*}Three replicate wells are required for each concentration. The detection range is 30 fg/μL~300pg/μL and this range can be expanded.

^{**}To reduce the number of repeat freeze-thaw and avoid contamination, it is recommended to store the control in aliquots at -25~-15°C for the first time.

^{***}Once thawed, DNA dilution buffer could be stored at 2~8°C for 7 days, if not used for a long time, please store at -25~-15°C.

^{****}Make sure the template is completely mixed, gently shake the mixture for 1 min for each gradient dilution.

2. Preparation of Test Sample (TS)

Prepare the test sample TS according to the experiment setup, as follows:

- 1) Take 100 μL of the test sample and add it to a 1.5 mL clean centrifuge tube. Label it as TS, perform sample pre-treatment, and prepare to purify the test sample.
- 2) To meet the requirement for analyzing four different extension lengths simultaneously, the amount of pre-treated test sample should be ≥120 μL. Therefore, it is recommended to prepare 2 tubes of each sample for pre-treatment, and after extraction, mix them together for use.

3. Negative Control Solution (NCS) preparation

Set the negative control in the experiment, the specific operation steps are as follows:

- 1) Add 100 μL sample matrix (or DNA dilution buffer) into a clean 1.5 mL tube, then marked as NCS.
- 2) Perform the DNA extraction of NCS sample together with the test samples to prepare the purified NCS sample.
- 3) To meet the requirement for analyzing four different extension lengths simultaneously, the amount of pre-treated NCS sample should be ≥120 μL. Therefore, it is recommended to prepare 2 tubes of each NCS sample for pre-treatment, and after extraction, mix them together for use.

4. No Template Control (NTC) preparation

Set the no template control in the experiment, the specific operation steps are as follows:

- 1) NTC requires no sample pretreatment, and can be configured at the stage of qPCR detection of residual DNA.
- 2) The NTC sample in each tube or well is 20 μ L Mix (i.e. 15 μ L HEK293 qPCR Mix + 5 μ L HEK293 Primer&probe Mix) + 10 μ L DNA Dilution Buffer. It is recommended to configure three replicate wells.

5. PCR reaction system

82 bp	Volume(μ L)
HEK293 qPCR Mix [*]	15
HEK293 Primer&Probe Mix-82	5
DNA Template	10
Total volume ^{**}	30

Table2 Reaction system for 82 bp fragment

133 bp	Volume(μ L)
HEK293 qPCR Mix [*]	15
HEK293 Primer&Probe Mix-133	5
DNA Template	10
Total volume ^{**}	30

Table3 Reaction system for 133 bp fragment

227 bp	Volume(μ L)
HEK293 qPCR Mix [*]	15
HEK293 Primer&Probe Mix-227	5
DNA Template	10
Total volume ^{**}	30

Table4 Reaction system for 227 bp fragment

515 bp	Volume(μ L)
HEK293 qPCR Mix [*]	15
HEK293 Primer&Probe Mix-515	5
DNA Template	10
Total volume ^{**}	30

Table5 Reaction system for 515 bp fragment

^{*}Calculate the total PCR reaction volume by the number of reactions: qPCR Mix =(the number of reactions+2) $\times$ (15+5) μ L (including the losses of two reaction wells). More than three replicates for each sample are recommended in the experiment.

^{**}After capping the tube or sealing the plate, centrifuge the reaction tube or plate at low speed for 10 secs. After sufficient shaking and mixing for 5 secs, repeat centrifuge to collect the liquid from the lid or wall to the bottom. Avoid bubbles during operation.

See below table for the recommended Plate setup:

	82 bp			133 bp			227 bp			515 bp		
	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std1	Std1	Std1	Std1	Std1	Std1	Std1	Std1	Std1	Std1	Std1
B	Std2	Std2	Std2	Std2	Std2	Std2	Std2	Std2	Std2	Std2	Std2	Std2
C	Std3	Std3	Std3	Std3	Std3	Std3	Std3	Std3	Std3	Std3	Std3	Std3
D	Std4	Std4	Std4	Std4	Std4	Std4	Std4	Std4	Std4	Std4	Std4	Std4

E	Std5	Std5	Std5	Std5	Std5	Std5	Std5	Std5	Std5	Std5	Std5	Std5
F	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
G	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS
H	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC

Table6 Computer-on reference board

This example shows the qPCR detection procedure for analyzing the amplification fragments of residual HEK293 DNA. The test samples include: 5 concentration gradients of HEK293 DNA standard curve, 1 test sample (TS), 1 negative control solution (NCS), and 1 no-template control (NTC). It is recommended to run 3 replicate wells for each sample.

6. Setup guidelines for a PCR Instrument (2-step method) (eg. Thermo ABI 7500 qPCR instrument, Version 2.0 software)

The following instructions apply only to Thermo ABI 7500 qPCR instrument (Software version 2.0). If you use a different instrument, refer to the applicable instrument guide for setup guidelines.

- 1) Create a new blank program and select "Absolute Quantification" as the detection template.
- 2) For the four different amplification fragment lengths, create new detection probes, naming them "HEK293-82", "HEK293-133", "HEK293-227", and "HEK293-515". Select the reporter fluorophore as "FAM" and the quencher fluorophore as "None". Set the reference dye for detection as "ROX" (the reference dye may be added or not depending on the instrument model and other factors).
- 3) In the "Assign target(s) to the selected wells" panel, set the "Task" field for the standard curve wells as "Standard", and assign the corresponding values in the "Quantity" field as "300000", "30000", "3000", "300", "30" (representing the DNA concentration per well, in fg/μL). Name the wells in the "Sample Name" field as "300 pg/μL", "30 pg/μL", "3 pg/μL", "300 fg/μL", "30 fg/μL". For the NTC wells, set the "Task" to "NTC". For the NCS and TS wells, set the "Task" to "Unknown", and name the wells "NCS" and "TS" in the "Sample Name" field. After setting these parameters, click "Start Run" to begin the instrument run.
- 4) Amplification program settings: Set the reaction volume of 30 μL.

Cycle Step	Temperature(°C)	Time	Cycles
Contaminant digestion	37°C	5 min	1
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	45
Annealing	60°C	30 sec	
Extension (Fluorescence collection)	72°C	30 sec	

Table7 Amplification procedure

7. Analysis of qPCR results

- 1) The system will automatically give the Threshold In the Amplification Plot panel of Analysis. The Threshold given by the system is too close to the baseline sometimes, resulting in a large difference in Ct between replicate wells. You can manually adjust the Threshold to an appropriate position and click Analyze. Then you can initially check whether the amplification curve is normal in Multicomponent Plot.
- 2) In the Result Analysis tab, review the Standard Curve plot. Verify the values for the R^2 , Efficiency, Slope and Y-intercept. For a normal standard curve, $R^2 > 0.99$, $90\% \leq \text{Eff\%} \leq 110\%$, $-3.6 \leq \text{Slope} \leq -3.1$.

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- 3) In the "View well table" pane in Analysis, the concentrations of each samples are showed in Quantity, the unit is fg/ μ L, the units can be convert in the assay report.
 - 4) The parameter settings of the result analysis need to be based on the specific model and the software version used, and can generally be automatically interpreted by the instrument.
 - 5) The Ct value of the negative control NCS should be greater than the mean of the lowest concentration Ct of the standard.
 - 6) The result of the no-template control (NTC) should be "Undetermined" or have a Ct value ≥ 32 .

Notes

1. This product is for research use only.
2. Please operate with lab coats and disposable gloves, for your safety.
3. Please read this manual carefully before using this reagent, and the experiment should be standardized, including sample handling, reaction system preparation and sample addition.
4. Ensure that each component is fully vortexized and centrifuged at low speed before use.