Vero Host Cell DNA Residue Detection Kit（2G）

Product description

Vero Host Cell DNA Residue Detection Kit is used for the quantitative analysis of Vero host cell DNA residuce in intermediate samples, semi-finished and finished products of various biological products.

This kit adopts Taqman fluorescent probe and the polymerase chain reaction (PCR) method, which has fg level minimum detection limit and can specifically and quickly detect the residual Vero cell DNA. The kit needs to be used together with the the Residual DNA Sample Preparation Kit (Cat# 18466ES).

Specifications

Cat.No.	41307ES50-EN / 41307ES60-EN
Size	50 T-EN / 100 T-EN

Components

Components No.	Name	41307ES50-EN	41307ES60-EN
41307-A	Vero qPCR Mix	0.75 mL	1.5 mL
41307-B	Vero Primer&Probe Mix	200 μL	400 μL
41307-C	DNA Dilution Buffer	2×1.8 mL	4×1.8 mL
41307-D	Vero DNA Control（30 ng/μL）	25 μL	50 μL
41307-E	IC*	50 μL	100 μL

*IC：Internal control.

Storage

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

Both 41307-A and 41307-B 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. Vero DNA Standard dilution and Standard curve preparation

The Vero DNA Control was gradient diluted using the DNA Dilution Buffer provided in the kit*, and the dilution concentration is 3 ng/μL, 300 pg/μL, 30 pg/μL, 3 pg/μL, 300 fg/μL, 30 fg/μL, 3 fg/μL,.

See detailed instructions below:

1) Thaw the Vero 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, Std6.

3) Add 90 μL DNA dilution buffer and 10 μL Vero 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
Std6	10 μL Std5 + 90 μL DNA Dilution Buffer	3 fg/μL

Table1 Standard gradient dilution

[*]Three replicate wells are required for each concentration.The detection range is 3 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 DNA 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 15 secs to 1 min for each gradient dilution.

2. Extraction Recovery Control (ERC) preparation

Set the concentration of Vero DNA in ERC as needed (the ERC sample was prepared with 30 pg Vero DNA as an example), as follows:

1) Add 100 μL test sample into a clean 1.5 mL tube, then add 10 μL 3pg/μL Vero DNA Standard (Std3) and mix well, marked as ERC.

2) Perform the DNA extraction of ERC sample together with the test samples to prepare the purified ERC sample.

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.

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 μL Mix (i.e. 15 μL Vero qPCR Mix + 4 μL Vero Primer&probe Mix + 1 μL IC) + 10 μL DNA Dilution Buffer. It is recommended to configure three replicate wells.

5. PCR reaction system

Component	Volume(μL)
Vero qPCR Mix[*]	15
Vero Primer&Probe Mix	4
IC	1
DNA Template	10
Total volume[**]	30

Table2 Reaction system

*Calculate the total PCR reaction volume by the number of reactions: qPCR Mix =(the number of reactions+2) × (15+4+1) μL (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:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC		TS 1	TS 1	TS 1		Std 1	Std 1	Std 1			
B	NTC		TS 2	TS 2	TS 2		Std 2	Std 2	Std 2			
C	NTC		TS 3	TS 3	TS 3		Std 3	Std 3	Std 3			
D							Std 4	Std 4	Std 4			
E	NCS		ERC 1	ERC 1	ERC 1		Std 5	Std 5	Std 5			
F	NCS		ERC 2	ERC 2	ERC 2		Std 6	Std 6	Std 6			
G	NCS		ERC 3	ERC 3	ERC 3							
H												

Table3 Computer-on reference board

The plate layout includes: 6 Std (the standard curve of 5 standard concentrations), 1 NTC (no template control), 1 NCS (negative control solution), 3 TS (test samples), 3 ERC (extraction recovery control). Three 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) Generate a new experiment, choose the template of absolute quantification or user-defined.

2) Create 1 detection probe, named "Vero-DNA", select reporter fluorophore as "FAM" and quench fluorophore as "None". ; create 1 more detection probe, name "IC" and select the reporter fluorophore as "CY5" and quenching fluorophore as "None". The reference fluorescence is ROX" (the reference fluorescence can be based on the instrument model, etc., select whether you need to add it).

3) In the 'Samples' pane, add all the samples information in turn. Then select the wells, choose the target and the samples correspondingly. Set the task of Vero DNA standard as standard, and assign the values 300000, 30000, 3000, 300, 30 , 3 (the unit of DNA concentration in each well is fg/μL) in the Quantity column, and name the wells Std 1, Std 2, Std 3, Std 4, Std 5, Std 6 correspondingly. Set the task of NTC as NTC. Set the NCS, TS, and ERC as Unknown, and named them according to the above Plate layout correspondingly. Then click next.

4) Set the amplification program: set the reaction volume as 30 μL.

Cycle Step	Temperature(°C)	Time	Cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	40

Annealing/Extension（Fluorescence collection）	60°C	30 sec	

Table4 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%≤Eff%≤110%, -3.6≤Slope≤-3.1.

3) In the ‘View well table’ pane in Analysis, the concentrations of each samples are showed in Quantity, the unit is fg/μL, 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) Calculate the spike recovery rate based on the test results of the sample TS to be measured and the sample spike recovery ERC, the recovery rate of spikes is required to be between 50%~150%.Spiked recovery rate meter formula: Recovery (%) = {Sample spiked assay (eg.pg/μL) - Sample assay (eg.pg/μL)} x Elution volume (μL) / Theoretical value of DNA addition amount (eg.pg) x 100%。

6) The Ct value of the negative control NCS should be greater than the mean of the lowest concentration Ct of the standard.

7) Template free control NTC should be Undetermined or Ct value ≥35.

8) The Ct-IC value of the sample to be tested should be consistent with or ±1 to the Ct-IC value of the NTC, and if the Ct-IC value of the sample to be tested is significantly larger compared to the Ct-IC value of the NTC, it indicates that the sample may be significantly inhibited. If spiked samples are tested at the same time, the sample spiked recovery results are prioritized and the IC results are used as a reference.

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.