Vero Host Cell DNA Residue Detection Kit

Application: Quantitative analysis of DNA residue in recombinant protein expressed products, purified intermediate and finished products from host cell.

For Research, Development and Manufacturing Use Only!

Not for Therapeutic Or Diagnostic Applications!

1. Introduction

This kit adopts Taqman probe fluorescence qPCR method. The kit has the advantages of high specificity and sensitivity by using specific primers & probes, LOQ can reach 1fg/ μ L level. This kit can be used in combination with our Magnetic Residual DNA Sample Preparation Kit (Cat#CG-DP100).

The preparation process of DNA Control is completely consistent with that of other host cell DNA national standards, therefore it has high purity and no protein and ion interference to ensure the accuracy of the sample quantitative detection.

The kit provides DNA Dilution Buffer, which enables good replicate parallelism in a single experiment and good reproducibility between multiple experiments.

2. Kit Components

DNA Amplification			
Components NO.	**Components Name**	**Cat#/Size**	
		VE-D050T(50T)	VE-D100T(100T)
B1	2XqPCR Mix	0.625mL	1.25mL
B2	Primer&Probe Mix	100μL	200μL
B3	DNA Dilution Buffer	2×1.5mL	4×1.5mL
B4	DNA Control (10ng/μL)	25μL	50μL
B5	RNase-Free H2O	0.5mL	1mL
B6	50X ROX Reference Dye(Optional)	0.15ml	0.3ml

**The usage of ROX reference dye is optional and depends on the type of instrument being used. Please refer to the details in PART 6*

3. Equipment Required But Not Provided

1.	Pipette: 5μL-1000μL	**5.**	Mini Centrifuge
2.	1.5/2ml RNase-/DNase-free Centrifuge Tube	**6.**	DNase/RNase-free 8-Tube Strip
3.	200uL RNase-/DNase-free PCR Tube	**7.**	Biological Safety Cabinet Class 2

4.	Vortex Mixer	**8.**	Fluorescence qPCR Detection System

4. Shipping and Storage

1) All components are shipped on dry ice.
2) The kit should be stored at -20℃ and it is recommended to be used within one year. B2 should be stored protected from light.
3) B2/B3/B4 can be stored at -20℃ for 2 years, while B1/B5/B6 can be stored at -20℃ for 1 year. B1/B5/B6 can also be purchased together as a separate set.

5. Preparation Before Experiment

1) Please read the manual thoroughly before use. All the components should be completely thawed, centrifuged at low speed, and vortexed well before use.
2) Avoiding the formation of bubbles, gently invert B1 (2XqPCR Mix) and B6 (50X ROX Reference Dye) after thawing them separately from -20° C storage. Use the solution only after complete homogenization. **Note**: If component B1and B6 will be frequently used for a period of time, they can be stored at 2-8° C for up to 3 months. Avoid repeated freeze-thaw cycles as much as possible. If not used after thawing, thoroughly mix before refreezing.
3) Do not pipette up and down repeatedly when mixing B2 (Primer & Probe Mix) and B4 (DNA Control) before use. Instead, a technique similar to cleaning the walls of the tube can be used to ensure the standard is evenly mixed. Note: To reduce the number of freeze-thaw cycles and avoid contamination, it is recommended to aliquot and store B4 (DNA Control) at -20° C upon first use.
4) The thawed but unused B3 (DNA Dilution Buffer) can be stored at 2-8° C for up to 7 days. If not used for an extended period, it should be stored at -20° C to maintain stability.
5) For your safety and health, please wear lab coat and disposable gloves when conducting the experiment. UV irradiation for 30 minutes before and after the experiment is recommended to eliminate potential DNA contamination in the environment.
6) Due to the high sensitivity of fluorescence quantitative PCR experiments, it is very important to maintain a clean working environment. Before starting the experiment, it is recommended to thoroughly clean the pipette and the surrounding work area, and remove any unnecessary items during the experiment.

6. ASSAY PROCEDURE

1) Dilution of DNA Control and Preparation of Standard Curves
   (1) Thaw B4 (DNA Control) and B3 (DNA Dilution Buffer) on ice, mix gently, and centrifuge at low speed for 10 seconds.
   (2) Take 7 clean 200μL PCR tubes, and label them as S0, S1, S2, S3, S4, S5 and S6. Add 45μL of DNA Dilution Buffer to each tube.

(3) Add 5μL of DNA Control (10 ng/μL) to S0 to dilute to 1000pg/ml. Centrifuge for 10 seconds, vortex for 5 seconds, then centrifuge again for 10 seconds. This concentration can be aliquoted and stored at -20℃ for short-term use (up to 3 months). Avoid repeated freeze-thaw cycles as much as possible.

(4) Dilution procedure in S1, S2, S3, S4, S5 and S6 tubes follows the same procedure as S0:

Tube	Dilution Process	Final Standard Concentration
S0	5μL DNA Control (10ng/μL) + 45 μL DNA Dilution Buffer	1000pg/μL
S1	5μL S0 + 45μL DNA Dilution Buffer	100pg/μL
S2	5μL S1 + 45μL DNA Dilution Buffer	10pg/μL
S3	5μL S2 + 45μL DNA Dilution Buffer	1pg/μL
S4	5μL S3 + 45μL DNA Dilution Buffer	100fg/μL
S5	5μL S4 + 45μL DNA Dilution Buffer	10fg/μL
S6	5μL S5 + 45μL DNA Dilution Buffer	1fg/μL

2) PCR Reaction System

Components	Volume(μL)
2XqPCR Mix	12.5
Primer&Probe Mix	2
DNA template（control or sample）	5
Add water	5.5
Total Volume	25

NOTES:

- Calculate the total volume of Mix solution required for this reaction based on the number of reaction wells: Mix solution = (number of reaction wells+4) * (12.5+2+5.5) μ L (including the volume lost in the 4 wells). It is recommended to perform the operation on ice.
- Standards and samples are recommended to be tested in triplicate. The detection range of the standard curve mentioned above is suitable for most experiments and can be adjusted as needed, such as 3fg/ μ L-300pg/ μ L.
- Maintain consistent experimental procedures. Seal the tube after sample addition, then centrifuge at low speed for 10 seconds to collect the liquid to the bottom. Vortex for at least 5 seconds to ensure complete mixing. Centrifuge again at low speed for 10 seconds. Remove the bubbles that may occurs.
- To ensure the accuracy of experimental results, we recommend diluting the protein concentration to 1-10mg/ml using 1X PBS for spike recovery experiments, which will ensure the recovery rate falls within

the range of 50% to 150%.

- Matching concentration for ROX Reference Dye of several instruments are listed in the table below.:

Instrument	Concentration
ABI PRISM 7000/7300/7700/7900HT/Step	2.5X（e.g. 1.25μL ROX/25μL system）
ABI 7500/7500Fast Stratagene Mx3000P/Mx3005P/Mx4000	0.5X（e.g. 0.25μL ROX/25μL system）
Roche/Bio-Rad/Eppendorf	No need to add

(1) Data Settings for PCR Instrument (Two-Step Method)

Phase	Temp.(℃)	Time	Content	Fluorescence Signal Collection	Cycles
Pre-denaturation	95℃	15min	Pre-denaturation	NO	1
PCR Reaction	95℃	3sec	Denaturation *	NO	40
	60℃	30sec	Annealing/Extension *	YES	

NOTES:

- The pre-denaturation condition for PCR reaction must be set at 95 ℃ for 15 minutes.
- Select Reporter as "FAM", Quencher as "TAMRA".
- Follow the instrument user manual for time setting on different models. Set the time as 1 sec. for ABI 7900HT/7900HT Fast/ViiA 7/StepOnePlus.
- Annealing/Extension *: Follow the instrument user manual for time setting on different models. The time settings for several common instruments are listed in the table below:

Instrument	Time Setting
ABI 7500 Fast/7900HT/7900HT Fast/ViiA 7/StepOne/StepOnePlus	30 sec
Roche LightCycle/LightCycle 480	20 sec
ABI 7000/7300	31 sec
ABI 7500	32 sec

7. Criteria for Results

1) Standard Curve: $R^2 > 0.99$; Amplification Efficiency: 90% ≤ Eff% ≤ 110%; Slope: -3.8~-3.1.
2) The recovery rate of spiked samples=(measured value of spiked samples - measured value of samples)/theoretical value of spiked samples * 100%, with a range of 50% -150%.
3) No Template Control (NTC): In the reaction system, replacing the target template with DNA Dilution Buffer while keeping other components unchanged, and the Ct value obtained should be 'Undetermined' or Ct value≥35.