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Plasmid DNA Residue (qPCR) Detection Kit

CATALOG #DK-P005, 100 Tests

INTRODUCTION

The plasmid residual DNA (qPCR) detection kit can rapidly and accurately quantitatively detect the residual detection of plasmid-dependent samples during the production process, such as lentiviruses, adenoviruses, mRNA vaccines and other biological products.

It is recommended to use KACTUS Host Cell Residual DNA (Magnetic Beads) Sample Preparation Kit (Catalog No. DK-SP001) for integrated workflow solutions.

KIT COMPONENTS

Components	Size	Storage Conditions
Plasmid Primer & Probe Mix	550 µL × 1 vial	-20°C, protected from light
2X qPCR Reaction Mix	1.6 mL × 1 vial	-20°C
Linearized DNA Quantitative Reference (1E8 copies/µL)	50 µL × 1 vial	
DNA Diluent	1.5 mL × 3 vials	

STORAGE AND SHELF LIFE

Unopened reagents maintain stability for 12 months from the date of manufacture when stored as recommended. After opening, please store the remaining reagents at -20°C. The reagents remain stable after 5 freeze-thaw cycles.

REQUIRED MATERIALS NOT SUPPLIED

- Real-time PCR instrument (ABI QuantStudio 3， ABI 7500, etc.), Centrifuge, Vortex
- Optical 8-tube strip or 96-well qPCR plate (sterile, nuclease-free and low retention), 1.5mL centrifuge tube (sterile and low retention)

WORKFLOW

Prepare the reagents and samples

1. **Prepare the kit.** Place all components on ice to ensure complete dissolution before use.
2. **Prepare DNA quantitative reference:**
   a) Vortex the Linearized DNA Quantitative Reference and DNA Diluent. Briefly centrifuge for 3-5 seconds.
   b) Dilute the Linearized DNA Quantitative Reference (1E8 copies/µL) as table below:

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Number	Final concentration (copies/μL)	Series dilution method	DNA Diluent
ST0	1E7	5 μL Linearized DNA Quantitative Reference (1E8 copies/μL)	45 μL
ST1	1E6	10 μL ST0	90 μL
ST2	1E5	10 μL ST1	90 μL
ST3	1E4	10 μL ST2	90 μL
ST4	1E3	10 μL ST3	90 μL
ST5	100	10 μL ST4	90 μL
ST6	10	10 μL ST5	90 μL

3. **Prepare the qPCR Working Mix**

   a) Calculate the required number of qPCR reaction wells using the following formula:

      i. Total wells N = (1 Non-Template Control + References ST1 to ST6 + Test Samples) × 2 or 3 Number of Replicates.

   b) Prepare the Working Mix per the table below.

      i. Total volume of Working Mix = 20 μL × (Total Wells N + 2)

Components	Volume Per Well
2X qPCR Reaction Mix	15 μL
Plasmid Primer & Probe Mix	5 μL
Total volume	20 μL

   c) **Prepare the qPCR reaction solution.** Place all the reagents on ice. Prepare the qPCR reaction solution per the table below:

	Sample	Reference ST1 to ST6	Non-Template Control (NTC)
Template	10 μL Sample	10 μL per ST	10 μL DNA Dilution
Working Mix per well	20 μL	20 μL	20 μL
Total Volume per well	30 μL	30 μL	30 μL

qPCR reaction parameters

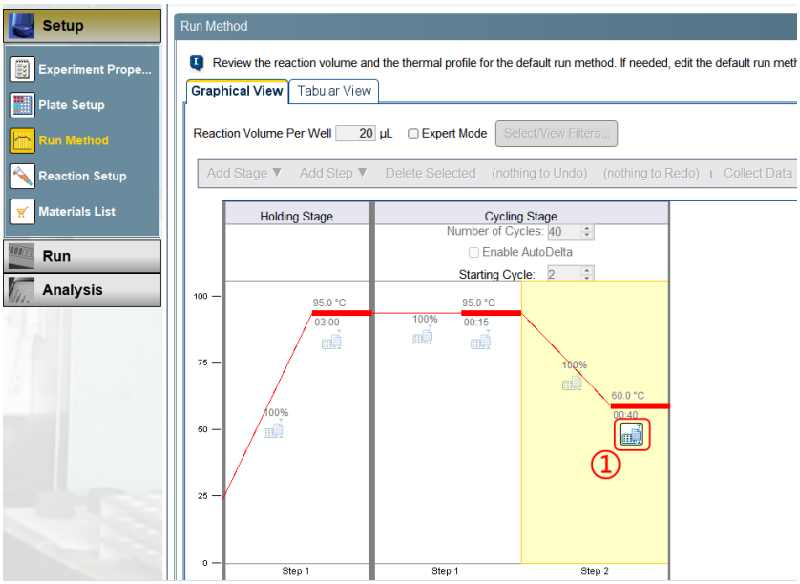
Using the American ABI 7500 Real-Time PCR System (version 2.06) as an example:

1. Create a blank new program. Experiment type: Quantitation-Standard Curve; Reagents: TaqMan® Reagents.
2. Use “Add New Targets” to create new probes and edit the probe names in “Target Name”. Select the desired fluorescent and quenching groups in “Reporter” and “Quencher”. “Reporter” is selected as FAM.

The “Quencher” is selected as NONE, the fluorescent group of internal control (IC) is selected as VIC, and the reference fluorescence is ROX.

3. Use “Add New Samples” to add new samples and name them in the corresponding Sample name column.
4. Set the reaction parameters. 60°C for 40 s in Stage 2 is set as fluorescence collection.

Stage 1	Pre-denaturation	Reps: 1	95°C	3 min
Stage 2	Cycle	Reps: 40	95°C	15 s
			60°C	40 s



5. Click “Start Run” to perform qPCR.

ANALYSIS

Using American ABI 7500 Real-Time PCR System (version 2.06) as an example:

1. Click “Analyze”. Check whether the shape of the amplification curve is normal.
2. Read the results in the “View Well Table” page. The unit is copies/µL.

NOTES

1. The components in the kit are stable and do not require aliquoting.
2. Prepare negative and positive samples in separate areas avoiding cross-contamination.
3. Always use clean pipette tips and avoid contamination.
4. Use the kit before expiration and do not mix different lots.
5. Thaw all components completely before use. Mix well and centrifuge briefly.
6. Follow instructions carefully for the best results.
7. Results depend on reagent quality, technique, and lab conditions.

8. Prepare enough samples for your needs.
9. For research use only. Not for medical diagnosis.

FAQs

Common Issues	Possible Reasons	Actions to Take
No Ct value	Incorrect fluorescence detection settings in the PCR program.	Ensure 60°C for 31 seconds in Stage 3 is set as fluorescence collection.
	Primer or probe degradation	Perform PAGE electrophoresis to determine if degradation had occurred.
	Template insufficient	Template degradation may be caused by impurities or damaged by repeated freeze-thaw.
Delayed Ct	qPCR reaction components degraded or are insufficient	Verify the ROX reference signal.
	Inhibitors in template	
Negative control shows a signal	Contamination	1. Repeat using newly prepared Working Mix. 2. Prepare the reaction solution in a laminar flow hood. 3. Separate the different experiment areas. Change to low-retention, nuclease-free tubes and pipette tips.
Abnormal amplification curve	1. High concentration of template; template degradation. 2. Evaporation or sealing issue; bubbles in tube	1. Dilute template. Retest with fresh reagents 2. Carefully transfer the reagents to avoid bubbles.

RELATED PRODUCTS

Product Name	Catalog No.
Host Cell Residual DNA (magnetic beads) Sample Preparation Kit	DK-SP001
Plasmid DNA Residue (qPCR) Detection Kit	DK-P005