Cat.: BP-QN120-100

Plasmid DNA Residue Detection Kit (qPCR) V01

Package Specification

100 Reactions / Kit

Intended Use

This kit is used for the quantitative detection of plasmid DNA residues in biological product samples.

Product Introduction

This kit adopts the PCR-fluorescent probe method, with primers and probes designed targeting the consensus sequences of plasmids, enabling quantitative detection of plasmid DNA residues in biological product samples.

The kit contains an exogenous internal control, which participates in nucleic acid extraction and amplification together with samples to monitor abnormalities in extraction and amplification and prevent false negatives.

The kit contains UDG enzyme to prevent contamination from amplification products.

Main Components

Components	Specification
Plasmid qPCR MIX	1.0 mL×2 Tubes
Plasmid Internal Control*	1.0 mL × 1 Tube
Plasmid Negative Control	1.0 mL×2 Tubes
Plasmid Calibrator ST1	0.5 mL × 1 Tube (5×10^1 copies/μL)
Plasmid Calibrator ST2	0.5 mL × 1 Tube (5×10^2 copies/μL)
Plasmid Calibrator ST3	0.5 mL × 1 Tube (5×10^3 copies/μL)
Plasmid Calibrator ST4	0.5 mL × 1 Tube (5×10^4 copies/μL)
Plasmid Calibrator ST5	0.5 mL × 1 Tube (5×10^5 copies/μL)
Plasmid Calibrator ST6	0.5 mL × 1 Tube (5×10^6 copies/μL)

Notes

Components from different batch numbers of the kit are not interchangeable.

Calibrators provided in the kit are linearized calibrators.

Reagents required for experiments but not included in the kit: Nucleic acid extraction or purification kit.

*If the internal control function is not used, no internal control is added during extraction, the CY5 channel is not set in the program, and CY5 results are ignored in result analysis.

*If the internal control function is used but no internal control is added during sample extraction, internal control can be added during amplification: add 1 μL internal control per reaction when preparing MIX, dispense 21 μL qPCR MIX per reaction, and the total PCR reaction volume is 31 μL per reaction.

Storage conditions and shelf life

1. Store in the dark at ≤ -20℃ with a validity period of 24 months.
2. Avoid repeated freeze-thaw cycles; the number of repeated freeze-thaw shall not exceed 10 times.
3. The product validity period and expiration date are indicated on the product label.

Applicable Instruments

Including but not limited to the following models: SLAN-96P, SLAN-96S Automatic Medical PCR Analysis System; ABI7500, ABI QuantStudio™ 5 Real-Time Fluorescent Quantitative PCR Instrument; Roche LightCycler 480 Fluorescent Quantitative PCR Instrument; Bio-Rad CFX96 Quantitative PCR Instrument.

Detection method

Take Plasmid qPCR MIX, Plasmid Internal Control, Plasmid Negative Control, and Plasmid Calibrators ST1–ST6 out of the kit, thaw at room temperature, mix thoroughly by vortexing, and centrifuge briefly for later use.

1. Preparation of Extraction and Recovery Control (ERC)

Set the plasmid spike concentration in ERC as needed (take preparation of ERC spiked with 5×10^6 copies as an example):

1)Add 100 μL of the sample into a clean 1.5 mL centrifuge tube;

2) Add 10 μL of ST5, mix well and centrifuge briefly, and label it as sample ERC.
3) Perform nucleic acid extraction and purification on Sample ERC together with the same batch of test samples to obtain Sample ERC nucleic acid.
2. Preparation of Reaction Mixture
2.1 Calculate the number of required PCR reactions based on the number of test specimens; 3 replicates per sample are recommended.
Number of PCR reactions = (6 calibrators + 1 NTC + 1 NCS + test samples + test sample ERC) × 3
Dispense the corresponding amount of PCR reaction mixture into a 96-well PCR plate or PCR 8-strip tubes at 20 μL per well.
2.2 Example of sample addition for each reaction well:

Component	Sample Volume
Standard Curve	20 μL Plasmid qPCR MIX + 10 μL ST1/ST2/ST3/ST4/ST5/ST6
NTC	20 μL Plasmid qPCR MIX + 10 μL Negative Control
NCS	20 μL Plasmid qPCR MIX + 10 μL NCS Purified Solution
Test Sample	20 μL Plasmid qPCR MIX + 10 μL Test Sample Purified Solution
Sample ERC	20 μL Plasmid qPCR MIX + 10 μL Sample ERC Purified Solution

3.Nucleic Acid Extraction of Samples
Follow the instructions of the nucleic acid extraction or purification kit; sample volume is 100 μL, internal control addition volume is 10 μL.
4.Fluorescent PCR Reaction
4.1 Add nucleic acid into Plasmid qPCR MIX according to Section 2.2, seal reaction tube caps or cover 96-well PCR plate with optical film, mix well, centrifuge briefly, and transfer to fluorescent PCR instrument.
4.2 Run the following program on the fluorescent PCR instrument:

Steps	Conditions	Cycles
UDG Treatment	50°C: 2 minutes	1
Pre-denaturation	95°C: 3 minutes	1
PCR Amplification	95°C: 10 seconds, 60°C (fluorescence collection): 30 seconds	45

Fluorescence channels: FAM (plasmid DNA), CY5 (plasmid internal control).For ABI series instruments, reference dye: ROX.
5.Result Determination
5.1 Threshold Setting
Adjust threshold based on instrument noise. Set to the mean fluorescence of cycles 3–15 plus 10× standard deviation, or to the maximum fluorescence of negative controls, above background fluctuation.
5.2 Internal Control Validation
For negative results, the Ct value of the Internal control should be ≤33; For strong positive results: internal control may show no value or poor value due to competitive inhibition.
5.3 Acceptance Criteria
1) Mean concentration of NTC and NCS ≤ 1 copy/μL, or mean Ct > mean Ct of the lowest calibrator.
2) Standard curve $R^2 \geq 0.98$, slope between −3.1 and −3.8.
3) Recovery of spiked samples (if applicable): 50% – 150%.
6.Result Analysis
6.1 SLAN-96P / SLAN-96S
1) Threshold adjustment (if needed): Go to Parameter Settings in the Experimental Analysis panel.
2) Plate setup: Set calibrator Sample Type to Standard, assign values (5×10^6 to 5×10^1 copies/μL) in Attributes, and name ST6–ST1.
3) View slope, intercept, correlation coefficient, and amplification efficiency in the Standard Curve panel.
4) Concentration reading: View concentration (copies/μL) in the Reaction Well Information Table; calculate spike recovery (50%–150%).
6.2 ABI 7500 (Software v2.4)
1) Threshold adjustment (if needed): Threshold: FAM = 0.1, CY5 = 0.02 in Amplification Plot.
2) Plate setup: Set calibrator Sample Type to Standard, assign values (5×10^6 to 5×10^1 copies/μL) in Attributes, and name

ST6–ST1. in Sample column. Set the Task of the NTC well to "NTC", set the Task of NCS wells, test sample wells and sample ERC wells to "Unknown", and name them NTC, NCS, S, ERC in the corresponding Sample Name column, then click "Analyze";
3) View slope, intercept, R^2, and amplification efficiency in “Standard Curve”.
4) Concentration reading: View quantity (copies/μL) in View Well Table; calculate spike recovery (50%–150%).

Precautions

1. Store the kit at -20℃ or below.
2. Read the manual carefully before use; follow the protocol strictly for optimal performance.
3. Use DNase/RNase-free consumables; process quickly and store frozen if not used immediately.
4. Do not use expired components or mix components from different batches.
5. Thaw reagents completely at room temperature and centrifuge briefly before use; avoid repeated freeze-thaw.
6. Use new disposable PE gloves for sealing; avoid bare-hand contact. Use powder-free, non-fluorescent latex gloves.
7. Follow strict PCR laboratory zoning: Reagent Prep → Extraction → Amplification → Analysis. Separate personnel, equipment, reagents, and airflow.
8. Cap samples and calibrators promptly after use to prevent contamination and false positives.
9. Do not open amplification tubes post-reaction. Dispose of waste properly outside the PCR laboratory.
10. Strong positive samples may suppress internal control signal due to competition.

Disclaimer

In all cases, the company's liability for this product is limited to the product's value itself.