Cat.: BP-QN21-100

Sf9 & AcNPV Residual DNA Detection Kit (qPCR) V01

Package Specification

100 Reactions / Kit

Intended Use

This kit is used for the quantitative detection of residual DNA from Sf9 cells and AcNPV in genetic engineering vaccines produced by the insect cell (Sf9)-baculovirus expression system.

Product Introduction

This kit adopts the PCR-fluorescent probe method, with primers and probes designed for the conserved genes of Sf9 cells and AcNPV respectively. It can quantitatively detect residual DNA from Sf9 cells and AcNPV in genetic engineering vaccines produced by the insect cell-baculovirus expression system.

The kit contains an internal control (IC) to monitor abnormalities in nucleic acid extraction and amplification, preventing false-negative results. It also contains UDG enzyme to prevent contamination from amplification products.

Main Components

Components	Specification
Sf9 & AcNPV qPCR MIX	1.0 mL×2 Tubes
Sf9 & AcNPV Internal control*	1.0 mL × 1 Tube
Sf9 & AcNPV Negative Control	1.0 mL×2 Tubes
Sf9 & AcNPV Calibrator ST1	0.5 mL × 1 Tube (Sf9: 3 fg/μL; AcNPV: 3 copies/μL)
Sf9 & AcNPV Calibrator ST2	0.5 mL × 1 Tube (Sf9: 30 fg/μL; AcNPV: 3×10^1 copies/μL)
Sf9 & AcNPV Calibrator ST3	0.5 mL × 1 Tube (Sf9: 300 fg/μL; AcNPV: 3×10^2 copies/μL)
Sf9 & AcNPV Calibrator ST4	0.5 mL × 1 Tube (Sf9: 3 pg/μL; AcNPV: 3×10^3 copies/μL)
Sf9 & AcNPV Calibrator ST5	0.5 mL × 1 Tube (Sf9: 30 pg/μL; AcNPV: 3×10^4 copies/μL)
Sf9 & AcNPV Calibrator ST6	0.5 mL × 1 Tube (Sf9: 300 pg/μL; AcNPV: 3×10^5 copies/μL)

Notes

Components in different batch numbers of reagent kits are not interchangeable.

Reagents required for experimental procedures but not included in the kit: nucleic acid extraction or purification kit.

*If the Internal control function in the kit is not used, no Internal control needs to be added during extraction. The Internal control channel may be omitted during program setup, and the Internal control results need not be considered during result analysis.

*If no Internal control is added during sample extraction but is introduced during amplification, 1 μL of Internal control should be added per reaction mixture when preparing the MIX. When aliquoting the qPCR MIX, use 21 μL per reaction, resulting in a total PCR reaction volume of 41 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 out Sf9 & AcNPV qPCR MIX, Sf9 & AcNPV Internal control, Sf9 & AcNPV Negative Control and Sf9 & AcNPV Calibrators ST1-ST6 from the kit, thaw at room temperature, fully vortex and mix well, then centrifuge briefly for later use.

1. Preparation of Extraction and Recovery Control (ERC)

Take preparation of ERC spiked with AcNPV at 3×10^4 copies as an example:

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

2) Add 10 μL of ST4, mix well and centrifuge briefly, and label it as sample ERC.
3) Extract and purify nucleic acid from Sample ERC together with test samples in the same run.
2. Preparation of Reaction Mixture
2.1 Calculate the required number of PCR reaction solutions according to the number of test samples, and it is generally recommended to set 3 replicates per sample.
Number of PCR reaction solutions = (6 concentration gradients of standard curve + 1 No Template Control (NTC) + 1 Negative Control Sample (NCS) + test samples + sample ERC) × 3. Then dispense the corresponding number of PCR reaction solutions into a 96-well PCR plate or PCR eight-tube strips at 20 μL per well.
2.2 Example of sample addition for each reaction well:

Component	Sample Volume
Standard Curve	20 μL Sf9 & AcNPV qPCR MIX + 20 μL ST1/ST2/ST3/ST4/ST5/ST6
NTC	20 μL Sf9 & AcNPV qPCR MIX + 20 μL Negative Control
NCS	20 μL Sf9 & AcNPV qPCR MIX + 20 μL NCS Purified Solution
Test Sample	20 μL Sf9 & AcNPV qPCR MIX + 20 μL Test Sample Purified Solution
Sample ERC	20 μL Sf9 & AcNPV qPCR MIX + 20 μL Sample ERC Purified Solution

3.Nucleic Acid Extraction of Samples
Follow the instruction manual of the nucleic acid extraction or purification kit for the operation steps, with a sample volume of 100μL and an Internal control addition volume of 10μL.
4.Fluorescent PCR Reaction
4.1 Add the nucleic acid to the Sf9 & AcNPV qPCR MIX according to the method in 2.2, cover the reaction tube caps or seal the 96-well PCR plate with an optical film, mix well, centrifuge briefly, and transfer to the 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 (Sf9), ROX (AcNPV), CY5 (Internal Control).For ABI instruments: set reference dye to none
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 ≤35; For positive results, the Internal control may have no value or poor value due to competitive inhibition.
5.3 Acceptance Criteria
1) NTC and NCS show no Ct 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 Taking SLAN-96P as an Example:
1) If the threshold needs to be adjusted, set the threshold to an appropriate level in the "Parameter Settings" of the "Experiment Analysis" panel;
2) In “Plate Editor”, set calibrators as “Standard” and assign concentrations: 3, 3×10^1, 3×10^2, 3×10^3, 3×10^4, 3×10^5 (fg/μL or copies/μL); label ST1–ST6.
3) View slope, intercept, R^2, and amplification efficiency in “Standard Curve”.
4) In the "Reaction Well Information Table" panel of "Experiment Analysis", the concentration value of the test result can be read in the "Concentration" column (unit:fg/μL or copies/μL). Calculate the spiked recovery rate based on the test results of the test sample and sample ERC, which is required to be between 50% and 150%.
6.2 Taking ABI 7500 qPCR Instrument (Software Version 2.4) as an Example:

1) If the threshold needs to be adjusted, set the Threshold of FAM, ROX, CY5 channel to an appropriate position in the Amplification Plot panel of the Analysis module;
2) In the Plate Setup panel of the Setup module, set the Task of the standard curve wells to "Standard", and assign concentrations: 3, 3×10^1, 3×10^2, 3×10^3, 3×10^4, 3×10^5 (fg/μL or copies/μL); label ST1–ST6. 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) In the View Well Table panel of the Analysis module, the concentration value of the test result can be read in the Quantity column (unit: fg/μL or copies/μL). Calculate the spiked recovery rate based on the test results of the test sample and sample ERC, which is required to be between 50% and 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.