248701 E. coli Host Cell Residual DNA Detection Kit

Product Introduction

This kit uses fluorescent probe qPCR to quantitatively detect residual E. coli DNA in recombinant proteins, antibodies, vaccines, and other biological products. It provides rapid detection with strong specificity.

Package Contents

Kit size: 100T

Code	Component	Specification
248701.1	E. coli DNA Positive Control	30 ng/μL, 50 μL × 1 vial
248701.2	Mixture of primers and probes	0.5 mL × 1 tube
248701.3	qPCR Master Mix	1.5 mL × 1 tube
248701.4	DNA Dilution Buffer	1.5 mL × 1 tube
248701.m	Instructions	1 copy

Quality Standards and Safety Information

Material	Quality Standard	Main Toxicity
E. coli DNA Positive Control	Not specified	Not specified
Mixture of primers and probes	Not specified	Not specified
qPCR Master Mix	Not specified	Not specified
DNA Dilution Buffer	Not specified	Not specified

Transportation and Storage

Transportation: Transport with ice packs.

Storage: Store at -20°C. Shelf life is two years.

Instructions for Use

1. Preparation of Positive Control Standard Curve Samples

The E. coli DNA positive control concentration is shown on the tube label. Confirm the actual concentration before dilution. Use the DNA dilution buffer provided in the kit to serially dilute the positive control to 3000 pg/μL, 300 pg/μL, 30 pg/μL, 3 pg/μL, 0.3 pg/μL, 0.03 pg/μL, and 0.003 pg/μL.

1. Remove the E. coli DNA positive control and DNA dilution buffer from -20°C and thaw on ice. After complete thawing, gently mix, then centrifuge briefly for 2 to 5 s.
2. Prepare 7 low-adsorption centrifuge tubes labeled ST0, ST1, ST2, ST3, ST4, ST5, and ST6. After each dilution step, vortex to mix and centrifuge briefly for 2 to 5 s before continuing to the next dilution.
3. After preparing the standard samples, store them at 2 to 8°C and use immediately.

Dilution Tube	Dilution Step	Concentration (pg/μL)
ST0	10 μL DNA positive control + 90 μL DNA diluent	3000
ST1	10 μL ST0 + 90 μL DNA diluent	300

Dilution Tube	Dilution Step	Concentration (pg/μL)
ST2	10 μL ST1 + 90 μL DNA diluent	30
ST3	10 μL ST2 + 90 μL DNA diluent	3
ST4	10 μL ST3 + 90 μL DNA diluent	0.3
ST5	10 μL ST4 + 90 μL DNA diluent	0.03
ST6	10 μL ST5 + 90 μL DNA diluent	0.003

Note: Thawed but unused DNA diluent may be temporarily stored at 2 to 8°C.

2. Preparation of Spike Recovery Control (ERC)

Set the E. coli DNA spike level for ERC. It is recommended to set the spike level at 2 to 30 times the sample's historical unspiked test value. The procedure below uses a 30 pg E. coli DNA spike as an example.

1. Add 100 μL of test sample to a 1.5 mL low-adsorption centrifuge tube.
2. Add 10 μL ST3, mix well, and label the tube as test sample ERC.
3. Pretreat ERC together with test samples from the same batch to prepare the ERC purified solution.

3. Preparation of Negative Control (NCS)

Set the negative control according to the experiment.

1. Add 100 μL of test sample matrix solution, or DNA diluent, to a 1.5 mL low-adsorption centrifuge tube and label it as NCS.
2. Pretreat NCS together with test samples from the same batch to prepare the NCS purified solution.

4. qPCR Reaction System

1. Calculate the required number of reaction wells:
   Number of reaction wells = (6 concentration-gradient standard curve samples + 1 blank control BLK + 1 negative quality control NCS + test sample + test sample ERC) × 3
2. Calculate reagent volumes for the run:
   Primer and probe mixture = (number of reaction wells + 2) × 5 μL
   qPCR Master Mix = (number of reaction wells + 2) × 15 μL
   The extra 2 wells are included as a loss allowance.
3. After all reagents reach room temperature, prepare the reaction mixture using the calculated amounts. Mix gently by vortexing, then load samples as shown below.

Sample Type	Loading per Well
Standard curve	20 μL reaction mixture + 10 μL ST1/ST2/ST3/ST4/ST5
BLK	20 μL reaction mixture + 10 μL DNA dilution solution
NCS	20 μL reaction mixture + 10 μL NCS purified solution
Test sample (SAM)	20 μL reaction mixture + 10 μL purified DNA test sample solution
Test sample ERC	20 μL reaction mixture + 10 μL ERC purified solution

5. qPCR Reaction Sample Loading

1. Use a 96-well PCR plate and add 20 μL reaction mixture to each required well first.
2. Add BLK, NCS, SAM, and ERC according to the loading plan. Then add 10 μL of the ST1, ST2, ST3, ST4, and ST5 DNA standard solutions. All samples are run in triplicate.
3. Seal the plate with adhesive film, centrifuge, and perform qPCR.

1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	BLK	BLK								
B	NCS	NCS	NCS	ST5	ST5	ST5					
C	SAM1	SAM1	SAM1	ST4	ST4	ST4					
D	SAM2	SAM2	SAM2	ST3	ST3	ST3					
E	SAM3	SAM3	SAM3	ST2	ST2	ST2					
F	ERC1	ERC1	ERC1	ST1	ST1	ST1					

1	2	3	4	5	6	7	8	9	10	11	12
G	ERC2	ERC2	ERC2								
H	ERC3	ERC3	ERC3								

6. qPCR Instrument Program Settings

1. Create a new blank program and select the absolute quantification assay template.
2. Create a new detection probe named E.coli-DNA. Set the reporter fluorophore to FAM, the quencher fluorophore to TAMRA, and the passive reference dye to ROX.
3. Set a two-step reaction program.
4. Program conditions: 95°C for 10 min; then 40 cycles of 95°C for 15 s and 60°C for 1 min.
5. Reaction volume: 30 μL.

Result Calculation

1. Test Sample Result

Exogenous DNA residual amount (pg/mg) = (dilution factor × mean value of the test sample (pg/μL) × elution volume (μL)) / (test sample protein concentration (mg/mL) × test sample extraction loading volume (mL))

Coefficient of variation (CV%) = (standard deviation of replicate well DNA content / mean value of replicate well DNA content) × 100%

If the DNA test result of the extraction sample is near the lower detection limit (ST5, Ct value ±2 cycles), or if the Ct result is greater than ST5, CV% is not calculated.

2. Recovery of Spiked Test Samples

Process the spiked sample in the same way as the test sample. Use the PCR standard curve equation and the Ct value of the spiked sample to calculate DNA content, then calculate spike recovery from the labeled spiked amount.

Spiked recovery % = ((test sample ERC concentration (pg/μL) - test sample concentration (pg/μL)) × elution volume (μL) / spiked amount (pg)) × 100%

Precautions

1. When using chemicals, wear an appropriate lab coat, disposable gloves, and safety goggles.
2. If the reagent is not used up at one time, store it in a -20°C freezer.
3. If reagent is accidentally splashed into the eyes, mouth, or nose, rinse immediately with plenty of clean water.
4. Do not use the kit if the label or test tube wall is stained, or if the writing is unclear.
5. Store the primer and probe mixture and qPCR Master Mix protected from light.
6. This product is for scientific research use by professionals only. It must not be used for clinical diagnosis or treatment, food, or drugs, and must not be stored in ordinary residences.
7. For safety and health, wear a lab coat and disposable gloves during operation.

Visual Reference