# Instructions for Use of *E.coli* Residual DNA Fragment Analysis Detection Kit (qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

**Cat. No. HG-EF001**

## Introduction

Based on fluorescent probe method, this kit quantitatively detects fragments of *E.coli* residual DNA with sizes of 62bp, 141bp, 284bp, and 517bp, respectively, covering the possible functional gene sizes to the greatest extent, with the sensitivity up to 3fg/μL, and can accurately quantify the residual amount and distribution of each fragment of *E. coli* DNA.
The kit is equipped with the sample preprocessing kit of our company (Cat. No.: HG-CL100) for sample preprocessing.
The detection range is from 3 fg/μL to $3 \times 10^5$ fg/μL.

## Specification

4 × 100 Reactions

## Kit components

Table 1: Kit components and storage conditions

| Components | Specification | Storage temperature |
|---|---|---|
| *E.coli* DNA quantitative reference（30ng/μL） | 50 μL x 1 vial | -20℃ |
| *E.coli* Primer&Probe MIX-62bp | 550 μL x 1 vial | |
| *E.coli* Primer&Probe MIX-141bp | 550 μL x 1 vial | |
| *E.coli* Primer&Probe MIX-284bp | 550 μL x 1 vial | |
| *E.coli* Primer&Probe MIX-517bp | 550 μL × 1 vial | |
| 2x qPCR Reaction Buffer | 1.6 mL × 4 vial | |
| DNA diluent | 1.5mL × 3 vial | |

## Product storage conditions and shelf life

See the above table for storage conditions, and the shelf life is 12 months. After opening, unused kits should be stored Under specified storage conditions.

## Applicable model (including but not limited to)

- ABI PRISM 7500
- CFX96(Bio-Rad)
- FQD-96A (Bioer)
- Roche Light Cycler 480

## Consumables and equipment to be self-prepared

Please prepare the following consumables and equipment before the test

- 1.5 mL or 2 mL sterile low adsorption centrifuge tubes
- 96-well qPCR plate or 8-strip tube adapted to PCR instrument
- 1000 μL, 200 μL, and 10 μL sterile low adsorption pipette tips with cartridge
- Fluorescence quantitative PCR instrument
- Pipettes of various specifications (e.g., 1000 μL, 200 μL, 10 μL, 2.5 μL)
- Centrifuge
- Oscillator
- Magnetic stand
- Water bath/metal bath

## Test steps

**Ⅰ. Sample Preprocessing**

Please refer to the operating instructions of our sample preprocessing kit (Cat. No.: HG-CL100) for details.

**Ⅱ. qPCR Operation Steps**

1. Preparation of quantitative reference and NTC/NSC

1.1 Quantitative reference: Take out DNA quantitative reference and DNA diluent, and thaw on ice; after thawing completely, shake gently to mix well, and centrifuge instantly;

1.2 Take 7 clean 1.5 mL centrifuge tubes and label them as ST0, ST1, ST2, ST3, ST4, ST5, and ST6, respectively;

1.3 The standard dilution process is shown in the table below:

Table 2

| Standard No. | Dilution volume | Concentration ( fg / μL) |
|---|---|---|
| ST0 | 10μL Quantitative reference + 90 μL DNA diluent | 3 x $10^6$ |
| ST1 | 10 μL ST0 + 90 μL DNA diluent | 3 x $10^5$ |
| ST2 | 10 μL ST1 + 90 μL DNA diluent | 3 x $10^4$ |
| ST3 | 10 μL ST2 + 90 μL DNA diluent | 3 x $10^3$ |
| ST4 | 10 μL ST3 + 90 μL DNA diluent | 3 x $10^2$ |
| ST5 | 10 μL ST4 + 90 μL DNA diluent | 3 x $10^1$ |
| ST6 | 10 μL ST5 + 90 μL DNA diluent | 3 x $10^0$ |

1.4 Preparation of NTC: 100 uL DNA diluent;

1.5 Preparation of NSC: Take 100 μL of DNA diluent and sample for sample preprocessing;

1.6 Spike recovery of ERC: It is suggested that 90 uL sample + 10 uL Quantitative Reference 3 can be prepared in other ways according to the actual situation.

2. Preparation and addition of qPCR reaction solution

2.1 Calculate the required number of reaction wells based on the numbers of standards and samples to be tested (generally, 3 replicate wells will be required for each sample):

Number of reaction wells = (Standard curve with 6 concentration gradients+ 1 negative control(NTC) +test sample) × 3

2.2 Calculate the total amount of qPCR MIX required for this time based on the number of reaction wells:

qPCR MIX = (Number of reaction wells + 2 or 3) × 20 μL (2 or 3 is operational loss)

2.3 Thaw the reagents to be used on ice, mix by gentle shaking, and prepare the qPCR MIX as shown in Table 3-6.

Table 3 MIX-62bp Preparation of qPCR Reaction

| Components | Volume required for single reaction |
|---|---|
| 2x qPCR Buffer | 15 |
| *E.coli* Primer&Probe MIX-62bp | 5 |
| Total volume | 20 |

Table 4 MIX-141bp Preparation of qPCR Reaction

| Components | Volume required for single reaction |
|---|---|
| 2x qPCR Buffer | 15 |
| *E.coli* Primer&Probe MIX-141bp | 5 |
| Total volume | 20 |

Table 5 MIX-284bp Preparation of qPCR Reaction

| Components | Volume required for single reaction |
|---|---|
| 2x qPCR Buffer | 15 |
| *E.coli* Primer&Probe MIX-284bp | 5 |
| Total volume | 20 |

Table 6 MIX-517bp Preparation of qPCR Reaction

| Components | Volume required for single reaction |
|---|---|
| 2x qPCR Buffer | 15 |
| *E.coli* Primer&Probe MIX-517bp | 5 |
| Total volume | 20 |

3. Thaw the required reagents on ice, mix well by gentle shaking, and load as shown in Table 7-10 (total volume of 30 μL):

Table 7 MIX-62bp Examples of loading to each reaction well

| | |
|---|---|
| **Standards** | 10μL quantitative reference 1~6 + MIX-62bp 20μL |
| **Negative control (NTC)** | 10μL DNA diluent + MIX-62bp 20μL |
| **Test sample** | 10μL test sample + MIX-62bp 20μL |

Table 8 MIX-141bp Examples of loading to each reaction well

| | |
|---|---|
| **Standards** | 10μL quantitative reference 1~6 + MIX-141bp 20μL |
| **Negative control (NTC)** | 10μL DNA diluent + MIX-141bp 20μL |
| **Test sample** | 10μL test sample + MIX-141bp 20μL |

Table 9 MIX-284bp Examples of loading to each reaction well

| | |
|---|---|
| **Standards** | 10μL quantitative reference 1~6 + MIX-284bp 20μL |
| **Negative control (NTC)** | 10μL DNA diluent + MIX-284bp 20μL |
| **Test sample** | 10μL test sample + MIX-284bp 20μL |

Table 10 MIX-517bp Examples of loading to each reaction well

| | |
|---|---|
| **Standards** | 10μL quantitative reference 1~6 + MIX-517bp 20μL |
| **Negative control (NTC)** | 10μL DNA diluent + MIX-517 bp 20μL |
| **Test sample** | 10μL test sample + MIX-517 bp 20μL |

4. In the experiment, sterile nuclease-free 8-tube strips or 96-well plates should be used for qPCR experiment, bubbles should be removed from the reaction system, and the liquid should be centrifuged to the bottom of the tube to prepare for the reaction.

5. Layout illustration of reaction wells

Table 11. Layout illustration of Plate

| | 62 bp | | | | | | 141 bp | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | ST1 | ST1 | ST1 | S1 | S1 | S1 | ST1 | ST1 | ST1 | S1 | S1 | S1 |
| B | ST2 | ST2 | ST2 | S2 | S2 | S2 | ST2 | ST2 | ST2 | S2 | S2 | S2 |
| C | ST3 | ST3 | ST3 | S3 | S3 | S3 | ST3 | ST3 | ST3 | S3 | S3 | S3 |
| D | ST4 | ST4 | ST4 | | | | ST4 | ST4 | ST4 | | | |
| E | ST5 | ST5 | ST5 | | | | ST5 | ST5 | ST5 | | | |
| F | ST6 | ST6 | ST6 | ERC -S1 | ERC -S1 | ERC -S1 | ST6 | ST6 | ST6 | ERC -S1 | ERC -S1 | ERC -S1 |
| G | NTC | NTC | NTC | ERC -S2 | ERC -S2 | ERC -S2 | NTC | NTC | NTC | ERC -S2 | ERC -S2 | ERC -S2 |
| H | NCS | NCS | NCS | ERC -S3 | ERC -S3 | ERC -S3 | NCS | NCS | NCS | ERC -S3 | ERC -S3 | ERC -S3 |

Table 12. Layout illustration of Plate

| | 284 bp | | | | | | 517 bp | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | ST1 | ST1 | ST1 | S1 | S1 | S1 | ST1 | ST1 | ST1 | S1 | S1 | S1 |
| B | ST2 | ST2 | ST2 | S2 | S2 | S2 | ST2 | ST2 | ST2 | S2 | S2 | S2 |
| C | ST3 | ST3 | ST3 | S3 | S3 | S3 | ST3 | ST3 | ST3 | S3 | S3 | S3 |
| D | ST4 | ST4 | ST4 | | | | ST4 | ST4 | ST4 | | | |
| E | ST5 | ST5 | ST5 | | | | ST5 | ST5 | ST5 | | | |
| F | ST6 | ST6 | ST6 | ERC -S1 | ERC -S1 | ERC -S1 | ST6 | ST6 | ST6 | ERC -S1 | ERC -S1 | ERC -S1 |
| G | NTC | NTC | NTC | ERC -S2 | ERC -S2 | ERC -S2 | NTC | NTC | NTC | ERC -S2 | ERC -S2 | ERC -S2 |
| H | NCS | NCS | NCS | ERC -S3 | ERC -S3 | ERC -S3 | NCS | NCS | NCS | ERC -S3 | ERC -S3 | ERC -S3 |

**III. qPCR reaction program and parameter setting**

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Create an experimental reaction program and set up a reaction program as shown in the table below.

Table 13. PCR reaction program

| | | | | |
|---|---|---|---|---|
| Stage1 | Contamination digestion | Reps : 1 | 50°C | 2min |
| Stage2 | Pre-denaturation | Reps : 1 | 95°C | 10min |
| Stage3 | Cyclic reaction | Reps : 40 | 95°C | 15s |
| | | | 56°C | 30s |
| | | | 72°C | 1min |

Note: The reaction volume is 30 μL. Set the program at 72°C for 1min for fluorescence collection; For other models of equipment, if you encounter any problems, you can consult our company or the instrument manufacturer.

2. Create an experimental reaction plate, click on "Select Fluorophores" and select fluorescent FAM. Select sample wells in the reaction plate chart, select "Unknown" in the "Sample Type" dropdown menu, check fluorescent FAM, and name the Target Name : different Mixes are named "62bp\141bp\284bp\517bp "; then input the number of replicate wells and Sample Name for each sample.

3. Select standard curve wells in the reaction plate chart, select "Standard" in the "Sample Type" dropdown menu, check fluorescent FAM, and name the Target Name : different Mixes are named "62bp\141bp\284bp\517bp; then input the number of replicate wells and Sample Name for each dilution gradient. Assign values of 3E+05, 3E+04, 3E+03, 3E+02, 3E+01, 3E+00 (in fg/μL), respectively, to the "Concentration" column of ST1, ST2, ST3, ST4, ST5, and ST6.
4. Click "Start Run" on the "Run" interface to perform PCR analysis.

**IV. qPCR result analysis**

Taking the CFX96 qPCR system (BIO-RAD) as an example.
1. Click Quantitation in Data Analysis Window to read the slope, intercept, amplification efficiency (Effect) and $R^2$ of the standard curve.
2. In the window Quantitation Data, the SQ Mean column reads the RCL test values of the no-template control (NTC) and the test sample in copies/μL.
3. Data reliability evaluation:

- The difference in Ct values between 3 replicate wells shall be less than 1.0, except for wells with Ct value greater than 35;
- The CT values of negative controls NTC and NCS should be greater than the CT value of the lowest concentration of the standard curve, or the criteria should be set based on the laboratory's own validation results;
- Linear correlation coefficient $R^2$ of the standard curve shall be equal or greater than 0.98, amplification efficiency shall be within 85%-110%;
- The recovery of ERC shall be within 50%-150%(spike recovery = ERC/(0.9*sample + 0.1*Quantitative Reference 3).

## Precautions

1. The kit has been validated for the stability (freezing-thawing and other factors) and does not require dispensing.
2. The preparation for negative samples and positive samples (reference and samples to be tested, etc.) should be separated into different environments and should not be operated in one area. The preparation personnel should wear neat masks, gloves and cleanroom garment.
3. Tips shall be changed between different loading steps in time to avoid cross-contamination and long-time opening.
4. The kit must be used within the shelf life.
5. All components in the kit are recommended to be used after melting in a low temperature environment.
6. The best detection effect can be ensured only by strictly following the instructions and using all the reagents provided with this kit.
7. Subsequent qPCR detection shall be performed immediately after sample preprocessing and purification as far as possible on the same day to ensure the accuracy of test results.
8. The final test results are closely related to the efficacy of reagents, operator's operation methods and test environment.
9. Our company is only responsible for the kit itself and not for the sample consumption caused by the use of the kit. Users should fully consider the possible usage of sample before use and reserve sufficient samples.
10. This kit is for in vitro research use only and is not used for clinical diagnosis.

## Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.


 

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