

Residual *E. coli* DNA Detection qPCR Kit

Description

The Residual *E. coli* DNA Detection qPCR Kit is designed for the sensitive detection of trace *E. coli* DNA in a sample using quantitative PCR (polymerase chain reaction). By using a specific primer and probe set that targets the 23S ribosomal RNA gene in *E. coli*, this kit can achieve a detection limit as low as 5 fg/ μ l while minimizing background noise. This kit provides enough 2x qPCR Master Mix (no dye) for 25 reactions and includes a positive control, dilution buffer, and ROX reference dye for generating reliable standard curves.

Background

The manufacture of biological drugs, including vaccines, antibodies, and recombinant proteins, is regulated by agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to ensure that clinical drugs do not contain harmful impurities. Most biologicals are prepared using host cell systems such as human cells, insect cells, or bacterial cells, and carry-over of host cell DNA must be kept to a minimum as it could compromise patient safety. Therefore, manufacturers must ensure that the amount of residual host cell DNA is kept within safety levels defined by regulatory agencies. While specific requirements may vary depending on the host cell type and route of administration, these agencies generally recommend a maximum limit of 10 ng of host cell DNA per drug dose.

To facilitate quality control, the Residual *E. coli* DNA Detection qPCR Kit enables the detection of *E. coli* DNA at concentrations as low as 5 fg/ μ l, with no cross-reactivity to mammalian cell DNA. The assay targets the bacterial gene encoding the 23S ribosomal RNA, which is present in seven copies per *E. coli* genome, allowing for highly sensitive detection. Although this gene is conserved among gram-negative bacteria, primer specificity has been validated by BLAST analysis to be exclusive to *E. coli*.

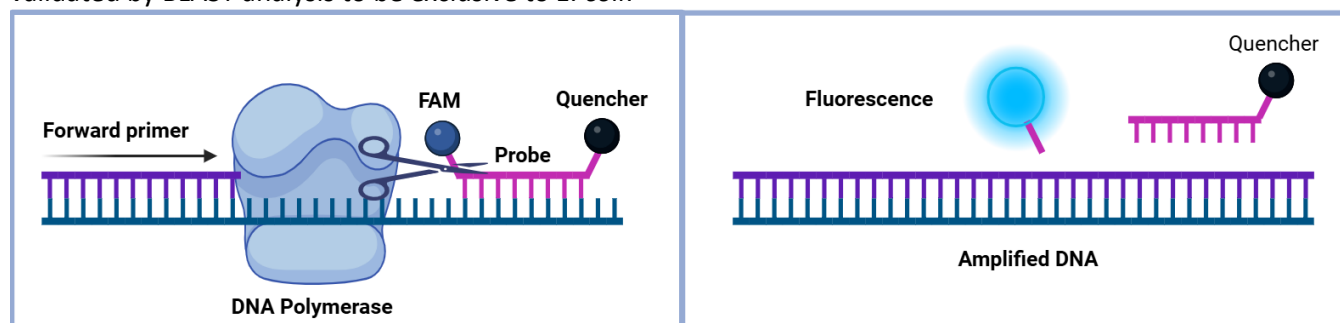


Figure 1. Principle of Probe-Based qPCR.

Detection utilizes a 6-FAM-labeled probe paired with Black Hole Quencher-1 (BHQ-1). (A) In the intact probe, fluorescence is quenched via Förster resonance energy transfer (FRET). (B) During the extension phase, the 5'-3' nuclease activity of Taq polymerase cleaves the probe, separating the 6-FAM fluorophore from the quencher to generate a fluorescent signal. The absorbance and emission maxima of 6-FAM are 495 nm and 520 nm, respectively.

Applications

- *E. coli* DNA quantification in samples of biologics such as antibodies or recombinant proteins produced in *E. coli*. Research Use only.

Supplied Materials

| Catalog # | Name | Amount | Storage |
|--------------------------|---------------------------------------|--------|---------|
| 93979-KC300 ⁺ | 2x qPCR Master Mix (no dye) | 300 µl | -20°C |
| 93980-KC60 ⁺ | <i>E. coli</i> Primer & Probe Mix | 60 µl | -20°C |
| 93981-KC10 ⁺ | <i>E. coli</i> DNA Control (10 ng/µl) | 10 µl | -20°C |
| 83737-KC400 ⁺ | Dilution Buffer | 400 µl | RT |
| 93982-KC250 ⁺ | DNA/RNase-Free Water | 250 µl | RT |
| 83580-KC10 ⁺ | ROX Dye (25 µM) | 10 µl | -20°C |

Storage Conditions

This kit will perform optimally for up to **6 months** from the date of receipt when the materials are stored as directed. We advise keeping the freeze-thaw cycles of the 2x qPCR Master Mix (no dye) to 5 cycles or less.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Materials Required but Not Supplied:

- CFX Connect Real-Time PCR Detection System (no probes required) or similar system (BioRad #1855201)
- 0.2 ml sterile qPCR tubes or 96 well qPCR plate
- 1.5 ml sterile microcentrifuge tubes
- Adjustable micropipette and sterile tips

Protocol**qPCR general guideline**

- Set up all reactions in duplicate and on ice.
- Limit light exposure of the *E. coli* Primer/Probe Mix and the ROX dye when in use.
- The suggested quantity of ROX Reference Dye to include in the Master Mix differs depending on the type of qPCR instrument you are using.
 - For equipment that does not require ROX: No dye is needed
 - For low ROX instruments: use 0.75 µl of ROX Reference Dye per 300 µl of Master Mix
 - For high ROX instruments: use 7.5 µl of ROX Reference Dye per 300 µl of Master Mix

| Reference Dye | PCR Instrument |
|------------------|--|
| Low ROX (30nM) | Applied Biosystems®: 7500, 7500 Fast, ViiA™7, Quant-Studio™ instruments Stratagene (Agilent): MX4000P, MX3000P, MX3005P |
| High ROX (300nM) | Applied Biosystems®: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™ |
| No ROX required | BioRad: iCycler™, MyiQ™, MiQ™ 2, iQ™ 5, CFX Opus, CFX-96 Touch™, CFX-384 Touch™ and Connect™, Chromo4™, MiniOpticon™ Qiagen: Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000 Eppendorf: Mastercycler® Realplex Illumina: Eco™ RealTime PCR System Cepheid: SmartCycler® Roche: LightCycler® 480, LightCycler® 2.0 |

- The assay should include *E. coli* DNA as the “Positive Control” and a “NTC” (No Template Control) to determine the limit of detection (LOD).

qPCR Set-up

- Prepare a serial dilution of *E. coli* DNA control following the table below. This will be used to generate the standard curve.

| Dilution Series | Volume of Control or previous dilution (µl) | Volume of Dilution Buffer (µl) | Concentration |
|-----------------|---|--------------------------------|---------------|
| Dilution 1 | 5 µl of <i>E. coli</i> DNA Control | 45 µl | 1 ng/µl |
| Dilution 2 | 5 µl of Dilution 1 | 45 µl | 100 pg/µl |
| Dilution 3 | 5 µl of Dilution 2 | 45 µl | 10 pg/µl |
| Dilution 4 | 5 µl of Dilution 3 | 45 µl | 1 pg/µl |
| Dilution 5 | 5 µl of Dilution 4 | 45 µl | 100 fg/µl |

- Set up the qPCR reactions with the 6 dilutions prepared and a No Template Control (NTC) in duplicate in a qPCR 200 µl tube or a 96-well PCR plate using the following volumes.

| Component | Test Sample | 1 ng | 100 pg | 10 pg | 1 pg | 100 fg | NTC |
|-----------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Test Sample | X µl | - | - | - | - | - | - |
| Dilution 1 | - | 1 µl | - | - | - | - | - |
| Dilution 2 | - | - | 1 µl | - | - | - | - |
| Dilution 3 | - | - | - | 1 µl | - | - | - |
| Dilution 4 | - | - | - | - | 1 µl | - | - |
| Dilution 5 | - | - | - | - | - | 1 µl | - |
| Dilution Buffer | - | - | - | - | - | - | 1 µl |
| Probe & Primer Mix | 2 µl | 2 µl | 2 µl | 2 µl | 2 µl | 2 µl | 2 µl |
| 2X qPCR Master Mix (No dye) | 10 µl | 10 µl | 10 µl | 10 µl | 10 µl | 10 µl | 10 µl |
| DNA/RNase-free water | 8 - X µl | 7 µl | 7 µl | 7 µl | 7 µl | 7 µl | 7 µl |
| Total | 20 µl | 20 µl | 20 µl | 20 µl | 20 µl | 20 µl | 20 µl |

3. Run qPCR reactions using the following parameters:

| Step | Temperature | Duration | Cycles |
|----------------------|-------------|------------|--------|
| Initial Denaturation | 95°C | 3 minutes | 1x |
| Denaturation | 95°C | 15 seconds | 40x |
| Annealing | 60°C | 30 seconds | |

* Capture FAM signal at the end of each annealing step.

Note: These parameters have been optimized for use with CFX Connect Real-Time PCR Detection System (Bio-Rad). Further optimization may be required for other instruments.

- Set the baseline threshold to a constant value above the background noise or use the auto-baseline feature to obtain Ct values.
- If the Ct value of your No Template Control is less than the Ct value of Dilution 5 (100 fg), do not use this data to calculate the amount of residual DNA in your test sample and repeat the experiment using a fresh serial dilution of the *E. coli* DNA control.

Data Analysis

- Plot the Ct value (Y-axis, linear scale) vs. *E. coli* DNA in fg (X-axis, logarithmic scale) for the *E. coli* DNA reference standard curve.
- Apply logarithmic regression to determine the amount of residual *E. coli* DNA in the unknown sample using the equation $y = mx + b$ from the logarithmic trendline equation you obtain from the standard curve.

Note: The R^2 value should be > 0.95 .

E. coli DNA = $e^{(Ct-b)/m}$, where **m** is the slope of the line and **b** is the y-intercept.

3. For example: if the trendline equation is $y = -3.57 \log(x) + 39.03$; **Ct** of test sample = 30.00, the amount of *E. coli* DNA (fg) is $= 10^{(30.00-39.03)/-3.30} = 338.39$ fg per reaction.
4. To calculate the amount of DNA per μl of test sample, one needs to account for the 20 μl reaction volume and the amount of test sample used for the reaction. Let's assume we used 2 μl of test sample:
 - Amount of *E. coli* DNA per μl of test sample = $\frac{[\text{Amount of DNA calculated for reaction (fg per reaction)}]}{[\text{Amount of test sample added } (\mu\text{l}) \times \text{reaction volume}(\mu\text{l})]}$
 - For Example:

$$\text{fg}/\mu\text{l} = \frac{(338.39 \text{ fg per reaction})}{(2 \mu\text{l} \times 20 \mu\text{l per reaction})}$$

$$\text{fg}/\mu\text{l} = 8.46 \text{ fg}/\mu\text{l of test sample}$$

Validation Data

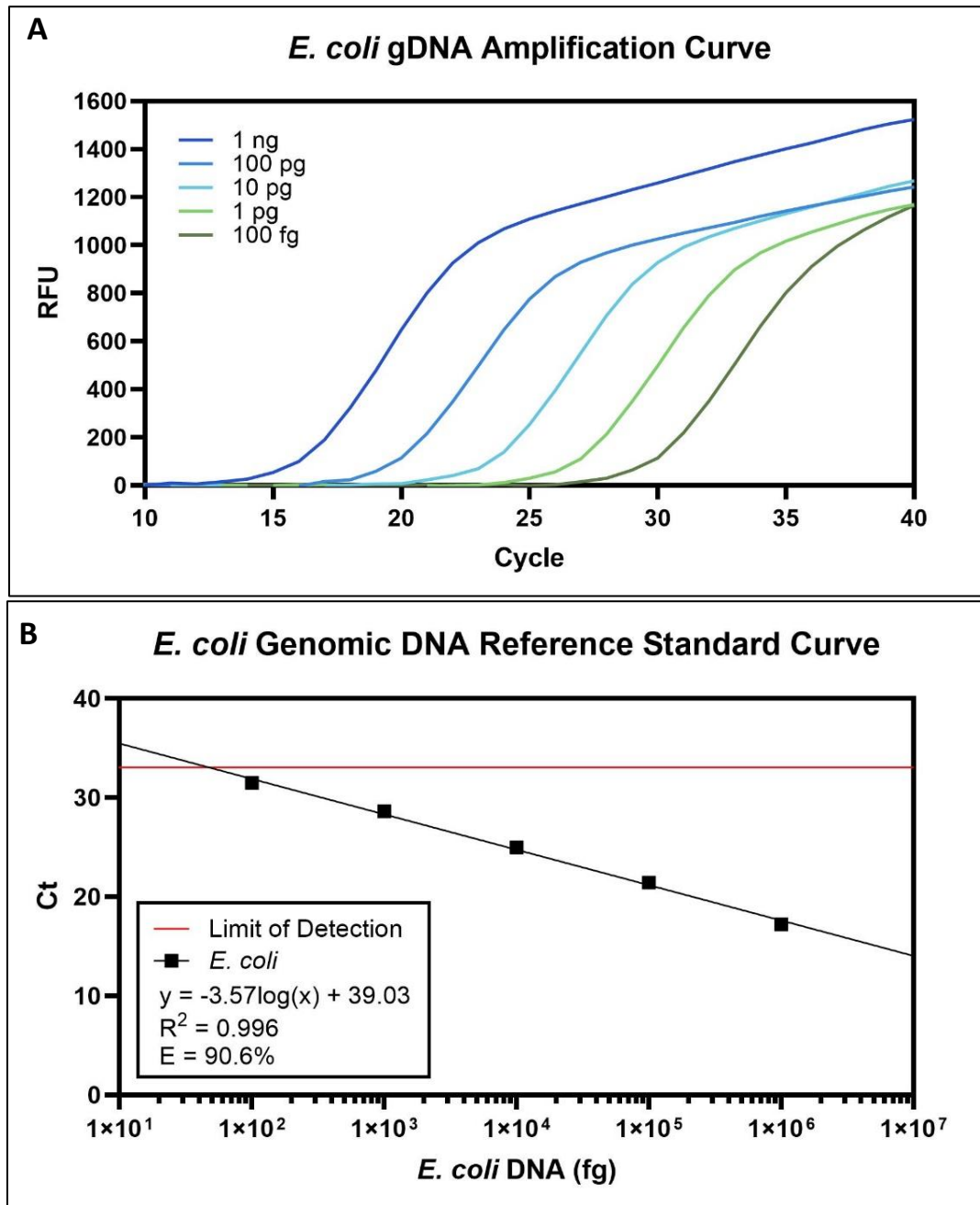


Figure 2. *E. coli* Genomic DNA Reference amplification and standard curves.

(A) qPCR results showing the amplification curves of a serial dilution of the *E. coli* Genomic DNA Reference in the 1 ng – 100 fg range, in triplicate. The qPCR was run with 23S Ribosomal RNA gene primers, a FAM/BHQ1 reporter probe, and a dye-free 2x qPCR mix. (B) A standard curve was generated using an average of the Ct values from the serial dilution and the logarithmic trendline equation and limit of detection are shown.

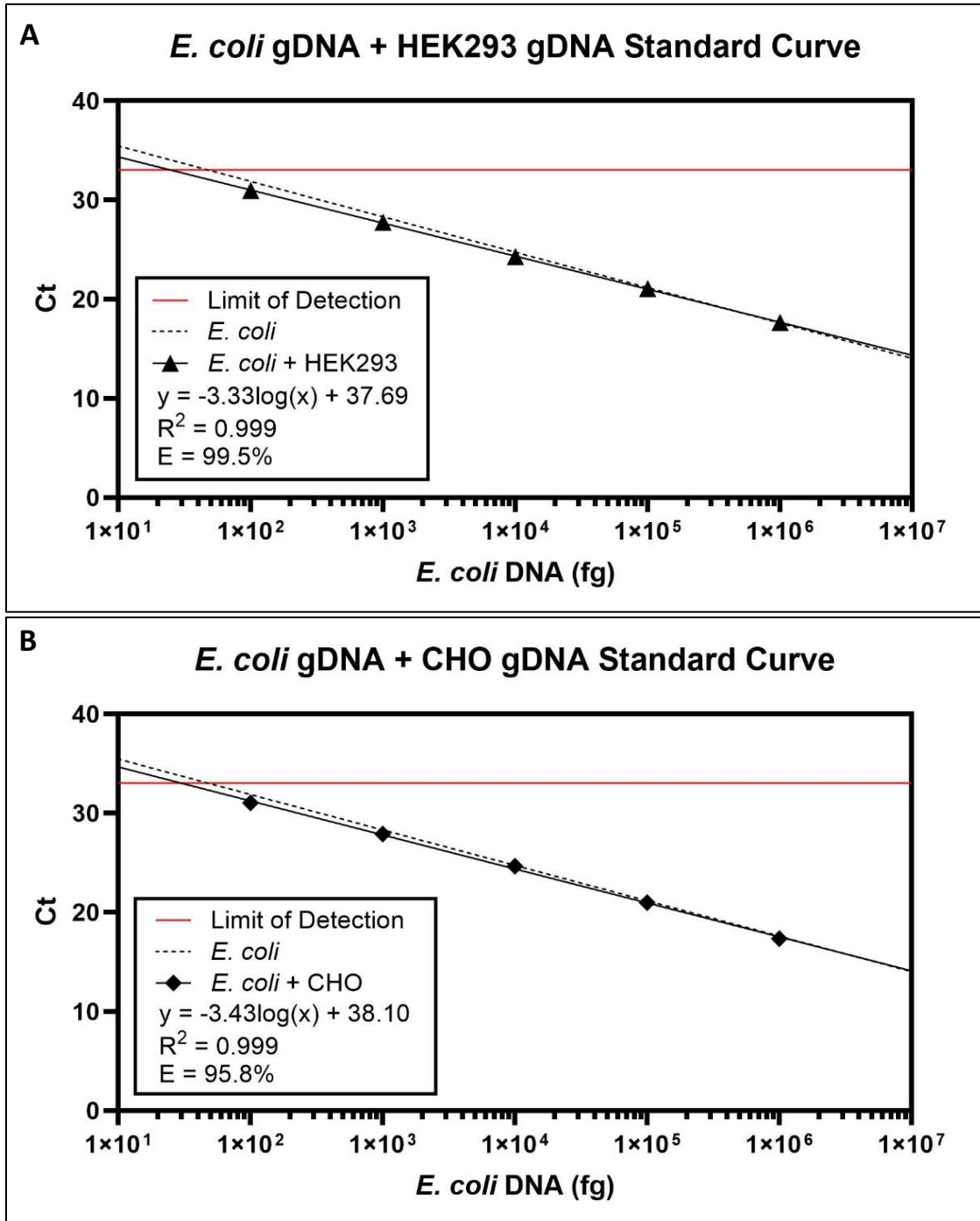


Figure 3. *E. coli* gDNA standard curve in the presence of HEK293 and CHO gDNA.

E. coli gDNA standard curves were generated from a serial dilution in the 1 ng – 100 fg range in the presence of 1 ng of HEK293 gDNA (A) or CHO gDNA (B). Results show no cross-reactivity with other DNA.

Data shown is representative.

Troubleshooting Guide

For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

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